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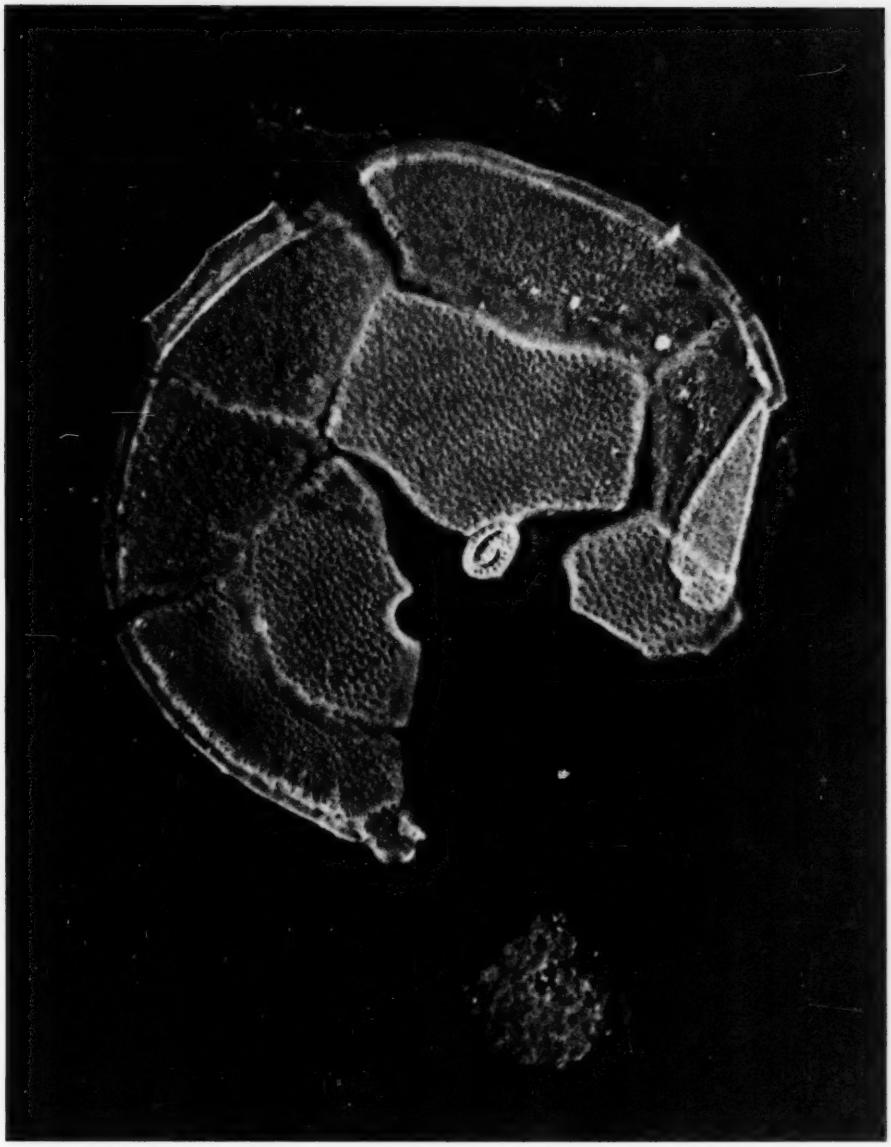
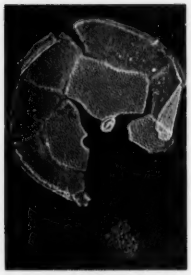


Gambierdiscus toxicus

Marine Fisheries REVIEW



On the cover: *Gambierdiscus toxicus* from the article by Alfred R. Loeblich III and Stephen R. Indelicato beginning on page 38.

Articles *48(4), 1986*

Departments

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Second International Conference on Ciguatera

Selected Papers Submitted to the Conference Held in San Juan, Puerto Rico, 23-25 April 1985

Preface

In the spring of 1982, a group of individuals with an interest in ciguatera met in San Juan, P.R., for two days under the auspices of the Caribbean Fisheries Management Council, the Sea Grant Program of the University of Puerto Rico, and the Corporation for the Development of Marine Resources (CODREMAR). Discussions centered on the socioeconomic impact of ciguatera as well as on strategies needed for resolving the accompanying problems. Those who could not attend the meeting were disappointed that no permanent record resulted from this conference.

By 1985, a second conference seemed timely in view of new investigators in the field and of the progress in ciguatera research. In addition, it would be possible to correct the shortcomings of the first meeting by publishing the proceedings of the second conference.

The Second Ciguatera Conference was held in the Condado Beach area of San Juan, P.R. Its success was, in large measure, due to the Caribbean Fisheries Management Council, whose staff and members gave generously of their time and resources. These Proceedings have resulted from planning and discussions between the Conference Steering and Editorial Committees.

Five sponsoring groups combined to support this venture: the Caribbean Fisheries Management Council (Omar Mùnoz-Roure, Executive Director), the Sea Grant Program and the Department of Marine Sciences of the University of Puerto Rico (Manuel Hernàndez-Avila, Director), CODREMAR (Ivan Sànchez-Ayèndec, Executive Director), the National Marine Fisheries Service Southeast Fisheries Center (Richard Berry, Director), and the Medical University of South Carolina. Each participant owes a special thanks to Omar Mùnoz-Roure, whose support and interest in ciguatera research made this conference possible.

Ciguatera research is multidisciplinary in nature and international in scope. Solutions have come slowly, but can be hastened by fostering greater awareness of the complex chemical and biological facets of ciguatera. Periodic conferences play an important part in free scientific discourse and in enhancing public understanding of all aspects of ciguatera—public health, scientific, and sociolegal. We look forward to further progress in this area and to additional opportunities to present the work in the field and in the laboratory to our colleagues for their information, for the opportunity to receive constructive criticism, and for all of us to continue to learn and understand this problem we call ciguatera.

T.H.
D.B.
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Ciguatera: A Legal and Social Overview

DAVID W. NELLIS and GEOFFREY W. BARNARD

ABSTRACT—Tropical marine fish constitute a major source of nutrition to the inhabitants of many developing nations of the Pacific and Caribbean regions. The occurrence of ciguatera, as well as the continual fear that ciguatoxin may be present in certain fish, limits the use of many potentially valuable species. Additionally, U.S. law may jeopardize the commercial value of suspect fish as seafood dealers acknowledge the civil liability risk associated with the sale of ciguatoxic fish. Overall, ciguatera results in the loss of a renewable resource and threatens the industry with potential liability suits when harvesting those elements of the marine food ecosystem that are marginally risky.

Introduction

Ciguatera is a disease produced in humans as the result of eating certain tropical marine fishes. Symptoms typically include nausea, vomiting, diarrhea, itching, and there may be a tingling or numbness in the mouth, which later spreads to the arms and legs. Often, cold objects seem hot and vice versa. Weakness and pain in the joints may be present. Initially, high blood pressure and tachycardia may be present, but in the progression of the disease, low blood pressure is common. In several cases, lowered blood pressure can require hospitalization and even produce death. Symptoms usually pass within weeks, but may linger for months.

Folklore

Some of the folklore regarding testing for ciguatera are:

1) Ciguatoxic fish have different coloration than normal fish: a) more yellow or brassy, b) stripes, c) darker.

2) Presence of isopod parasites indicates nonciguatoxic fish.

3) Raw flesh of ciguatoxic fish, especially the liver, tastes bitter or hot in the mouth.

4) Flies will not land on exposed flesh of ciguatoxic fish.

5) Silver or sweet potato turns black when boiled with ciguatoxic fish.

6) Ciguatoxic fish have a brassy or coppery odor.

7) Ciguatoxic fish have enlarged or bloated stomachs.

8) Ciguatoxic fish have yellow mucous or yellow inner linings of the gullet.

9) Ciguatoxic fish have a green tint to raw flesh.

10) Suspected species with roe are ciguatoxic.

11) Ants will not eat ciguatoxic fish.

12) Ciguatoxic fish have tiny black "veins" running throughout the flesh.

But experience has demonstrated that none of these can be considered reliable.

However, a test is available to the layman which is inconvenient but effective. The flesh of a suspect fish may be fed to a cat or mongoose because they respond to ciguatoxin similarly to man. The viscera of fishes (especially the liver) has higher concentrations of ciguatoxin than the flesh (Yasumoto and Scheuer, 1969). Using the liver rather than muscle tissue for ciguatera testing thus increases the sensitivity of this crude but effective test. In the laboratory, a quantitive test for ciguatoxin is to observe the response to the injection of a serially diluted flesh extract into a mouse.

In recent years it has been strongly suggested that ciguatoxin is derived from a toxin elaborated by the dinoflagellate *Gambierdiscus toxicus* (Bagnis et al., 1980). The dinoflagellate has typically been found living among sessile macroalgae or attached to dead coral detritus or rocks in shallow water. Presumably, herbivorous fish eat the algae from the rocks and accumulate the toxin in their tissues. Carnivorous fish consuming the herbivores subsequently concentrate the toxin.

Historically, folklore belief is that ciguatera originates in areas of copper concentrations, either in natural outcrops or on the copper sheathing of sunken vessels. The copper is believed to promote the growth of a certain type of "sea moss." This concept may have some basis in fact in that a sunken vessel may provide an ecological environment which favors the colonizing sessile algae that in turn would support *G. toxicus*.

Since ciguatoxin is chemically stable and is accumulated throughout the life of the fish, higher concentrations may occur in larger fish. This has led to another piece of folklore: "An individual fish that is less than 10 percent of the maximum size attained by the species is usually safe."

Analogously, species high in the food chain or older in age may carry larger amounts of toxin. However, individual food preference and availability can greatly alter these generalities. Conceivably, a large carnivorous fish may contain no significant toxin, while a small

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omnivore may produce a clinical case of poisoning. Similarly, certain species, because of their dietary preferences, may pose a higher toxicity risk, while closely allied species do not.

The uneven, patchy distribution of the organism in the environment and of the toxin in the ecosystem can lead to complex considerations in predicting the toxicity of a food fish. The north side of St. Thomas, Virgin Islands, is considered to be safe from ciguatera, but the south side has a high incidence in certain species. Meanwhile, St. Croix, 40 miles to the south, is generally free of ciguatera. The dog snapper, *Lutjanus jocu*, is typically encountered on coral reefs and is regularly associated with ciguatera, while the mutton snapper, *L. analis*, seen on the same reefs and sand flats, is deemed to be safe from ciguatera. The dog snapper includes algae-eating fish in its diet, while the mutton snapper consumes sand-dwelling invertebrates.

A physical or chemical test for the presence of toxin has been unsuccessfully sought for over 20 years. The concentration of toxin present in the most toxic fish is extremely small. The challenge of detecting this molecule is made more difficult by the lack of the toxin available to researchers. Total toxin purified by all researchers to date has been less than 0.002 g. Even with this small amount of material, the ciguatoxin molecule has been found to be a long chain polyether with hydroxyl groups and a molecular weight of 1111 (Nukina et al., 1984). Investigators have been attempting the difficult task of developing a test for a still unidentified chemical that is only available in microgram amounts after laborious extractions.

Because of the continued absence of a convenient, reliable test, a careful but also fatalistic attitude is held by native seafood consumers in areas with ciguatera: One should be careful about species, size, and geographic origin of a fish, but an occasional ciguatera intoxication is accepted as inevitable.

Legal Considerations

Since the turn of the century when pure food and drug legislation was enacted, such as the Federal Food, Drug, and Cosmetic Act of 1906, 34 Stat. 768, Americans have come to expect the highest standards in goods sold for human consumption.

The Uniform Commercial Code, followed by most of the states and adopted in the Virgin Islands in 1965, provides in Section 2-314 in pertinent part,

> (1) . . .a warranty that the goods shall be merchantable is implied in a contract for their sale if the seller is a merchant with respect to goods of that kind. Under this section the serving for value of food or drink to be consumed either on the premises or elsewhere is a sale.

The Restatement of Torts, Second, Section 402A(2)a, also in effect in the Virgin Islands, imposes strict liability on a seller of food, even though he may have "exercised all possible care in the preparation and sale of his product." This concept developed from the old English law which imposed criminal penalties on suppliers of "corrupt" food and drink.

Within the context of strict liability, the courts have struggled with the proposition that a seller of ciguatoxic fish may be held responsible, even though he has exercised all possible care in the purchase and preparation of the fish and could not in any event have determined whether the fish was toxic.

To resolve this tension in the law between strict liability and the inability to determine toxicity despite the exercise of all possible care, the courts have focused on the consumer's knowledge of the ciguatoxic potential of the fish and have considered whether fish poisoning was within the reasonable expectation of the consumer or whether the consumer knowingly assumed the risk.

Much of the litigation related to ciguatera fish poisoning has taken place in the District Court of the Virgin Islands. No cases have been found in the jurisprudence of Puerto Rico, though attorneys licensed in that jurisdiction report that results would be similar to those in the Virgin Islands.

In a case decided in 1968, shortly after the adoption of the Uniform Commercial Code in the Islands, the plaintiff contracted ciguatera fish poisoning when she ate a platter of fish at the defendant's restaurant. The evidence disclosed that the plaintiff, a long-time resident of St. Croix, was aware that, occasionally, persons eating fresh, local fish are poisoned, and the jury, finding that she knowingly assumed the risk, found in favor of the restaurant. Judge Maris, in sustaining the jury's verdict in *Bronson* vs. *Club Comanche Inc.* 6 V.I. 683, 286 F. Supp. 21 (D.C.V.I. 1968) observed that:

> (T)he form of contributory negligence which consists in voluntarily and unreasonably proceeding to encounter an unknown danger, and which is commonly called assumption of risk, may be a defense in a case of strict liability such as this. If the consumer is fully aware of the danger and nevertheless proceeds voluntarily to make use of the product and is injured by it he is barred from recovery.

The defense of assumption of the risk was also cited with approval in the later case of *Hoch* vs. *Venture Enterprise Inc.*, 473 F. Supp.541 (D.C.V.I. 1979), where it was determined that full factual development of all pertinent considerations would be required for the jury to resolve the issue.

Another approach was suggested by Chief Judge Christian in the case of *Battiste* vs. *St. Thomas Diving Club*, 1979 St. Thomas Supp. 164 (D.C.V.I. 1979). In this case the court examined two lines of authority for dealing with injuries suffered from consuming food in a restaurant, the "foreign-natural" test and the "reasonable expectations" test.

Under the "foreign-natural" test, the presence of substances that are natural to the ingredients, such as a piece of oyster shell in oyster stew, does not breach the vendor's implied warranty that the food is wholesome and fit. The court, however, preferred the "reasonable expectations" test because it permits the jury to determine whether the consumer could reasonably expect to find the toxic substance in the fish.

Of greater interest are the court's observations in *Battiste*, *supra* with respect to the public policy considerations.

> This discussion leads the Court to the public policy arguments raised by defendant. Certainly, the continued serving of local fish by Virgin Islands restaurants is a desirable economic and gustatory goal. Unfortunately, the Court cannot ignore the competing concern that patrons of our restaurants should be afforded notice of the risks inherent in the consumption of local delicacies. The alternative is the restaurants which, however small, are in the better position to spread the risk, must bear the risk. Perhaps the most facile solution would be for menus to contain a warning about the possibility of fish poisoning. This is not a particularly appetizing suggestion, but it would solve the problem of notice at little cost to the restaurant. It can hardly be argued that such a warning would substantially injure the local fish industry since it is a fair statement that many restaurant patrons today order local fish despite their awareness of danger of fish poisoning. The warning would simply insure that all patrons have the benefit of such knowledge.

In order to insulate the valuable local fishing industry from liability, it would seem fundamentally important to ensure that a form of warning be used at every link of the commercial chain, from the fisherman to the wholesaler to the retailer and/or the restaurant. In Puerto Rico, there are prominently posted warnings at places where fish are sold pursuant to the act creating the Corporation for the Development and Administrator of the Marine, Lacustrine, and Fluvial Resources, 12 L.P.R.A. Section 1351. It would also seem prudent that the insurer insist on such warnings before underwriting the business risk.

Future litigation will no doubt address the adequacy of the warning, relative to which species are suspect and the frequency of ciguatera poisoning in certain species. Nonetheless, the use of warnings would substantially reduce the likelihood of an adverse verdict and a high award of damages in favor of the unsuspecting tourist.

The major Virgin Islands insurance underwriters are not particularly concerned over the liability associated with ciguatera in restaurants. The agents usually determine that restaurants use "safe" species of fish from areas with low ciguatera hazard. While the agents recognize the value of a warning on menus, they accede to the restaurant's concern that a warning would adversely influence sales. One insurance agent required a warning sign on the premises and a warning notice on invoices of a fish market, which has since closed. A second fish market posted a warning sign but removed it after observing adverse customer response. Insurance adjustors and underwriters typically settle ciguatera poisoning claims for small amounts rather than taking the issue before the courts.

In international trade, the Food, Drug and Cosmetic Act requires that food importers prove the safety and wholesomeness of foodstuffs entering the United States. Federal agencies have not yet addressed the question of importing potentially ciguatoxic fish. With increasing populations of Caribbean peoples residing in the United States and encouragement of trade via the Caribbean Basin Initiative, it is likely that tropical marine fishes with potential for ciguatera will be increasingly imported to the United States.

The impact of ciguatera on a small island society is significant (Olsen et al., 1984). Tacket[1] estimated that the annual incidence of fish poisonings reported to the emergency room in St. Thomas, Virgin Islands, was around 4.2 cases per thousand population. In a household survey, she reported a level of 7.3 per thousand, indicating that 43 percent of the cases are not reported to the emergency room. McMillan et al. (1980) found from a Virgin Islands telephone survey that 22 percent of all households surveyed experienced at least one poisoning in 5 years. Taylor (cited by Tacket[1]) reported that this figure was as high as 31 percent in homes where fish was eaten. Surveys of ciguatera incidence are subject to considerable bias. Many of the households in which large amounts of local fish are consumed do not have telephones, and local people familiar with ciguatera generally do not seek professional medical assistance unless the symptoms become critical. In this regard, St. Thomas is probably typical of other islands where ciguatera is a normally accepted risk associated with the eating of local fish. Consumers who prepare fish at home attempt to reduce their risk by selecting certain species, certain sizes, and reliable vendors.

In a series of 48 interviews with fishermen, most of those interviewed in the northern U.S. Virgin Islands said they avoided fishing certain areas due to ciguatera. On St. Croix, only one fisherman restricted his fishing areas. However, fishermen always claimed they did not alter their fishing methods due to concern for ciguatera. In the northern Virgin Islands, all fishermen released certain fish that could be ciguatoxic, mainly barracuda and amberjack. Thirty-eight percent of the fishermen on St. Croix released fish at some time due to ciguatera concern.

No interviewed fisherman declined to capture certain abundant but potentially ciguatoxic fish. Thus, kingfish would be caught and marketed from an area of known ciguatera incidence. Suspect ciguatoxic yellowfin grouper are caught in large numbers when they become easily susceptible to capture in breeding aggregations. When asked if some customers avoided certain fish due to ciguatera concerns, all but one of the fishermen said yes. When asked to predict what percentage their annual income could be increased by a sure test for ciguatera, the response ranged from 0 to 75 percent.

Although the fish marketing industry is small in the Virgin Islands, ciguatera is of major concern to all who sell local fish. All businesses avoid selling certain fish (barracuda, amberjack, cubera, and large dog snappers) and buy only from reliable sources.

All vendors claimed to be willing to talk freely with customers about ciguatera. They expressed concern over legal liabilities associated with the sale of ciguatoxic fish and about loss of customers due to possible ciguatera. All businesses claimed they would change their present marketing practices if a test

[1]Tacket, C. Studies of epidemiological and clinical aspects of ciguatera. Unpubl. presentation, 1981 Ciguatera Conference, San Juan, P.R. Center for Disease Control, Atlanta, Ga.

for ciguatera was available. Their willingness to pay for a ciguatera test varied from $1.00 to $6.00 per fish. Of 12 restaurants contacted, all selected only certain species of fish from selected vendors. Two of the restaurants had a policy of preparing only imported fish that were judged to be without risk.

All of the above considerations, however, result in underutilization of a significant renewable fishery resource. The fish highly suspected for ciguatera are also of the greatest potential commercial and recreational value. Large snappers, groupers, and barracuda are eagerly sought by sport fishermen and divers and are highly esteemed at the dining table.

When large predatory fish high in the food chain are not harvested due to ciguatera, they in turn compete with fishermen for other harvestable fish that would otherwise be available to consumers.

A simple, inexpensive test for the presence of ciguatoxin would enhance the developing fishing industries of many tropical nations and allow use of a presently untapped renewable resource.

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Epidemiology and Impact of Ciguatera in the Pacific: A Review

NANCY DAVIS LEWIS

Introduction

Ciguatera, first described in the Caribbean (Martyr, 1555), has a long recorded history in the Pacific as well. Sailors with the Spanish explorer, de Quiros, suffered from ciguatera in Vanuatu in 1606 (Dalrymple, 1770), and Anderson (1776) described an intoxication aboard one of Cook's ships, the *Resolution*, in the same island group. The pantropical distribution of ciguatera and the fact that the fish are unaffected by the toxin(s) suggest that it has existed far longer than the historical record indicates.

Ciguatera is a significant health and resource problem in tropical areas, largely because of its erratic and often unpredictable spatial and temporal distribution. The eating of toxic fish remains a risk for both seafarers and tourists, but it is a much more pronounced problem for the inhabitants of tropical islands, who depend on the resources of the seas for food and livelihood. The epidemiology of ciguatera in the Pacific has received considerable attention (Banner and Helfrich, 1964; Halstead, 1978; Bagnis, 1976, 1977; Lewis, 1981, 1984a, b). In this paper, the epidemiology is updated through 1983-84, and some considerations for health, nutrition, and specifically marine resource development are presented. Banner (1976), Withers (1982), and Baden (1983) have reviewed the etiology, chemistry, pharmacology, and ecology.

ABSTRACT—For inhabitants of the Pacific Islands, the mean reported incidence of ciguatera from 1979 to 1983 was 97/100,000. As a health problem, ciguatera also appears to be increasing on some Pacific islands that had formerly experienced lower levels of endemicity. Ciguatera also has important implications for nutrition and the development of inshore fishery resources. The epidemiology of ciguatera in the Pacific is presented, and viewing ciguatera as a phenomenon to which islanders have had to adapt for centuries, the implications for health, nutrition, and resource development are discussed.

Epidemiology

The most consistent, albeit incomplete, information on the existence of cigatoxic fish in the Pacific is from morbidity reports. Intensive investigations of the distribution of both *Gambierdiscus toxicus* and toxic fish have been made at specific sites, but these are limited in both time and extent (Bagnis, 1969, 1973a, 1977; Yasumoto et al., 1979, 1984). Morbidity for selected diseases for the Pacific Island Region (not including Hawaii and Australia) is recorded by the South Pacific Commission's South Pacific Epidemiological and Health Information Service (SPEHIS) (South Pacific Commission, 1973-84). Ciguatera incidence for 1973-83 is presented in Table 1. The region is composed of 21 island states and territories (Fig. 1), and there are a host of cultural, economic, and practical factors that influence reporting. For example, sample surveys suggest a wide range (9-75 percent) in the number of individuals who go to a western medical facility when experiencing ciguatera (Bagnis, 1973a; Dawson, 1977; Lewis, 1981). There are often considerable differences between a country's central register of disease and the statistics reported to SPEHIS. In Fiji, Naryan (1980) indicated that there were 791 cases of ciguatera reported to the Ministry of Health between 1975-78. SPEHIS, based on reports submitted to it, listed 449.

The reported annual incidence of ciguatera for the 11 years from 1973 to 1983 was 97/100,000 for the region as a whole. Using the conservative estimate that this represents 20 percent of actual incidence, then actual incidence would be 500/100,000. The regional rate has been quite constant over the 11-year period, with 1973, 1979, and 1983 being years with marginally fewer cases reported. Lawrence et al. (1980) estimated that in Miami the actual incidence of ciguatera was 5/10,000 (50/100,000), making the estimated incidence for The Pacific ten times that of Miami.

Looking at the number of cases and mean rates for individual countries for the last 5 years of the period (1979-83) (Fig. 2), we see that some have reported annual morbidity rates several times that of the region. French Polynesia (585/100,000) had an average annual rate six times that of the region as a whole, and Tuvalu (484/100,000), five times. Kiribati (462/100,000) also had a mean incidence rate almost five times as high as the region as a whole. The reported rate for Tokelau, 1,338/100,000, was exceptionally high.

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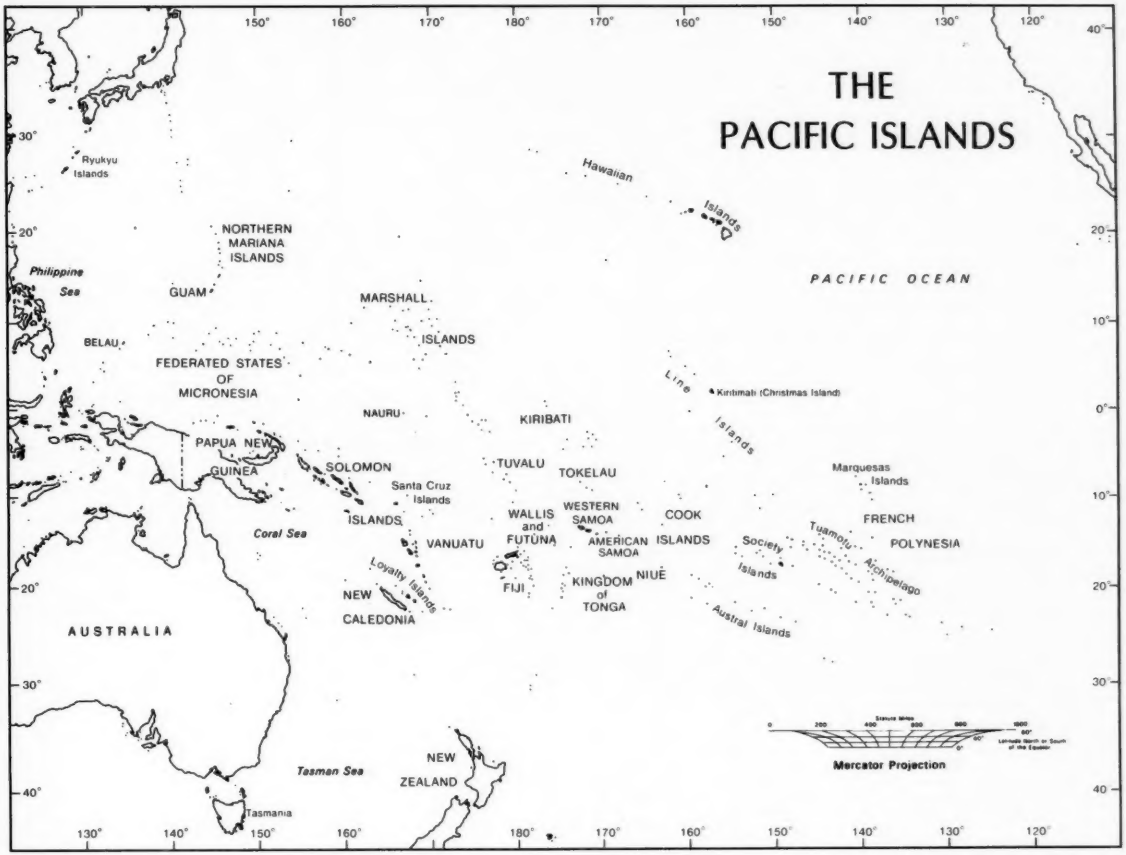

Figure 1.—The Pacific Islands.

Table 1.—Ciguatera morbidity, South Pacific Region, 1973-83, as reported to South Pacific Epidemiological and Health Information Service.

Country	Number of cases													Rate per 100,000 1973-83	Rate per 100,000 1979-83
	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	Total	Mean		
American Samoa	4	0	0	0	0	0	70	30	31	97	69	301	27.36	87	179
Cook Islands	0	0	0	0	0	0	0	1	2	0	0	3	0.27	1	3
Fiji	6	26	150	29	69	201	131	265	123	71	0	1,071	97.36	16	18
French Polynesia	607	867	625	660	502	821	677	937	1,145	831	789	8,461	769.18	545	585
Guam	0	0	21	16	6	6	9	0	4	3	21	86	7.82	8	7
Kiribati	101	175	187	77	41	38	78	187	286	418	414	2,002	182.00	324	462
Nauru	0	0	0	0	0	0	1	5	0	0	0	6	0.55	7	15
New Caledonia	0	200	518	647	487	488	188	147	107	130	112	3,024	274.91	200	96
Niue	7	1	35	4	0	0	0	3	3	0	0	53	4.82	130	38
Papua New Guinea[1]	0	0	16	0	0	0	0	0	0	0	0	16	1.45	<1	<1
Solomon Islands	1	7	0	7	6	6	0	4	4	0	2	37	3.36	2	1
Tokelau	0	0	0	8	0	0	14	0	3	17	73	115	10.45	653	1,338
Tonga	11	58	12	17	43	13	8	7	2	29	14	214	19.45	21	12
TIPI	240	264	208	313	326	296	191	217	163	119	120	2,455	223.18	173	114
Tuvalu	0	0	0	49	44	71	21	27	73	47	16	348	31.64	439	484
Vanuatu	0	0	35	28	50	53	67	0	32	12	0	277	25.18	25	19
Wallis and Futuna	0	0	3	7	0	0	0	0	0	0	0	10	0.91	9	0
Western Samoa	65	89	15	17	81	179	62	115	127	98	59	907	82.45	54	59
Total	1,042	1,687	1,825	1,879	1,655	2,172	1,517	1,945	2,105	1,870	1,689	19,386	1,762.36	97.1	96.1

[1]Papua New Guinea has been excluded from the regional analysis.

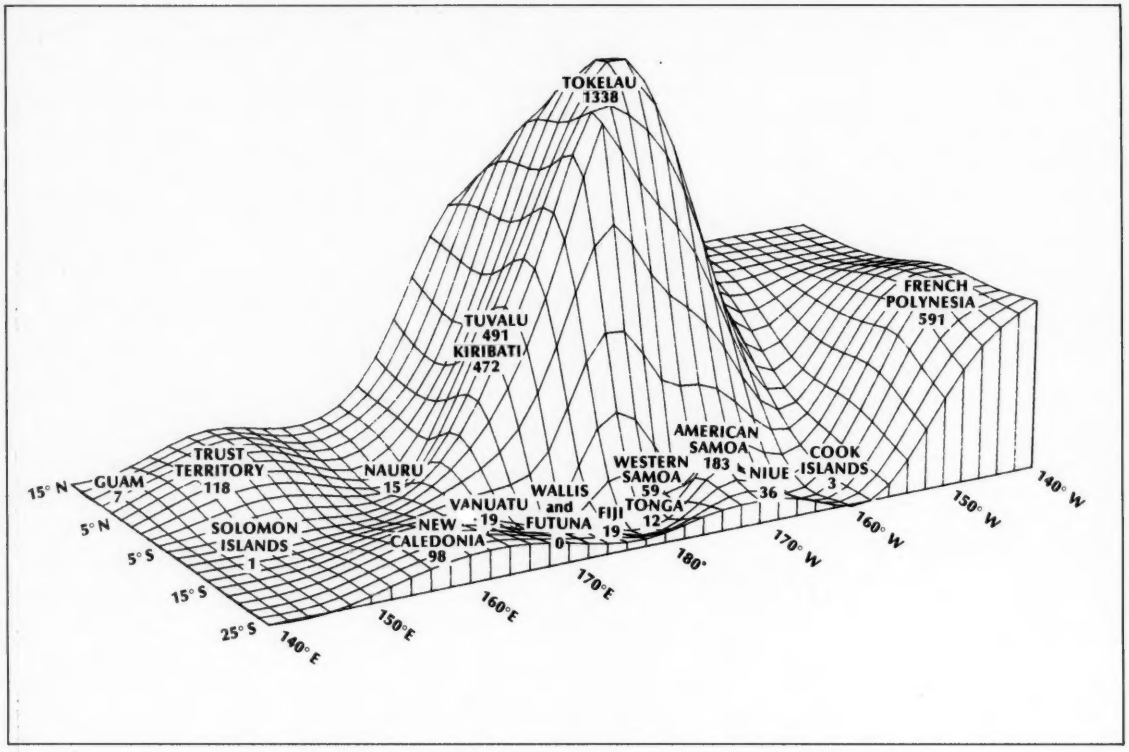

Figure 2.—Mean annual incidence of ciguatera (cases per 100,000), 1979-83.

Various patterns emerge in the three subregions of Oceania, Polynesia, Micronesia, and Melanesia. Incidence in French Polynesia has been consistently high throughout the period with a peak in 1981. While the longstanding research efforts of Raymond Bagnis and his colleagues at the Institut Recherches Medicales Louis Malardé in Papeete may have led to increased reporting, evidence suggests that ciguatera is a more serious problem in French Polynesia than in some other parts of the Pacific. The island groups within French Polynesia exhibit different levels of toxicity. It has been highest in the Gambiers, but this is not reflected in the morbidity statistics because of avoidance of locally caught fish. Species incriminated in the other groups are listed in Table 2. Disastrous hurricanes hit French Polynesia in 1982-83 and because of the long acknowledged association between disruption of marine substrate and increases in ciguatera, researchers are monitoring for increases in the causative organism. Islands to the west in Polynesia exhibit a less clear picture; the Cook Islands are in many respects a ciguatera anomaly with very low rates of ciguatera. Tonga also has relatively low rates of ciguatera. In Samoa, toxic fish were reported soon after the turn of the 20th century (Jordan, 1902; Spear, 1904) and the toxicity has persisted, but not at high levels. The species responsible (Table 2) are primarily carnivores. It is in the Tokelau that ciguatera seems to be most serious. Tokelau is a tiny territory of New Zealand made up of three coral atolls with a population of 1,600 and land area of only 10 km^2. The incidence rate was 1,338/100,000 for the period 1979-83. For 1983 alone the incidence was 4,867/100,000 and partial data from 1984 indicate an even higher rate. The seriousness of this, particularly in the last 2 years, which showed a precipitous increase, is obvious for this small population. They are isolated and highly dependent on the resources of the sea, and they are also distant from hospital facilities that can treat severe cases. Tuvalu is another small Pacific country with elevated rates of ciguatera, 484/100,000 annually from 1979 to 1983, based on a population of 7,600. On the single island nation of Niue, ciguatera was a more serious problem following World War II (Bagnis, 1973b), but ciguatera has been declining in recent years as it has in the French Territory of

Table 2.—Species perceived as commonly toxic in selected Pacific islands (Lewis, 1984b).

Species	Marquesas					Tuamotus				Societies				Samoa			Other				
	Hiva Oa	Tahuata	Fatu Hiva	Nuku Hiva	Ua Pou	Ua Huka	Takaroa	Manihi	Rangiroa	Other	Tahiti	Moorea	Huahine	Raiatea	Bora Bora	Tutuila	Upolu	Savai'i	Tonga	Fiji	Vanuatu	New Caled.	Hawaii
Carcharhinidae																☆	☆	☆	★				
Clupeidae																☆	☆	☆	★	★		★	★
Lycodontis sp.	●	●	●	●	●	●	□	□				■			■	☆	☆		★	★	★	★	
Sphyraena barracuda	●		●		●	●	□	□	□	□	■	■		■		☆	☆	☆	★	★	★	★	★
S. fosteri							□									☆	☆		★				
Gymnosarda unicolor		●		●							■										★	★	
Mugilidae											■								★				★
Acanthocybium solandri																						★	
Scomberomorus commersoni																			★	★	★		
Caranx sp.			●	●			□			□		■	■	■	■	☆	☆		★	★	★	★	★
Seriola dumerili																							★
Plectropoma maculatum																						★	
P. leopardus							□	□	□	□	■	■		■	■								
P. melanoleucus																						★	
Variola louti			●											■					★	★	★	★	
Serranidae																☆	☆	☆					
Cephalopholis argus	●		●	●			□	□	□			■	■	■	■				★	★	★		
Epinephelus tauvina	●		●				□	□		□		■		■									
E. merra	●	●												■					★				
E. microdon												■									★	★	
E. fasciatus	●																						
Epinephelus sp.																☆	☆	☆	★			★	★
Lutjanus monostigma			●	●	●		□	□			■	■	■	■	■	☆			★	★			
L. bohar	●	●	●	●	●	●	□	□	□	□	■	■	■	■	■	☆	☆	☆	★	★	★	★	
L. rivulatus											■	■							★				
L. argentimaculatus																	☆	☆	★		★	★	
L. gibbus	●	●	●	●	●		□	□			■	■		■		☆	☆		★				
L. fluviflamma																					★	★	
Lutjanus sp.																							★
Aphareus furcatus																							★
Aprion virescens			●							□													★
Monotaxis grandoculis				●	●	●	□	□				■										★	★
Gnathodentx aurolineatus	●	●	●	●			□	□															
Diagramma pictum																					★	★	
Lethrinus miniatus	●	●	●								■	■		■	■							★	
Lethrinus sp.									□							☆		☆	★	★	★		
Mullidae																☆							★
Cheilinus undualtus							□	□	□	□	■			■	■								
Cheilinus sp.																							★
Scaridae	●	●	●	●	●		□	□	□	□	■			■	■								★
Ctenochaetus cynoguttatus	●	●	●	●	●																		
C. striatus							□	□	□	□	■	■	■	■	■								
Acanthurus olivaceus				●																			
A. achilles				●																			
A. lineatus		●	●																				
Acanthurus sp.																							★
Naso brevirostris	●		●																				
N. herri	●		●																				
Balistidae	●	●	●	●	●	●	□	□	□	□	■	■		■	■				★			★	
Balistoides viridescens							□	□	□														
Arothron sp.																☆		☆					

Table 3—Ciguatera breakdown for Trust Territory of the Pacific Islands, 1982-83.

Islands	No. of cases 1982	No. of cases 1983	1982-83 rate per 100,000
Federated States of Micronesia	0	4	2
Marshalls Islands	100	85	282
Commonwealth of the Northern Marianas	17	31	130
Palau	0	0	0
Total	117	120	81

Wallis and Futuna (Table 1).

In Micronesia, ciguatera was a problem for both Japanese and American forces during World War II, and this focused scientific attention on ciguatera. Until 1982, statistics, for what is now the Trust Territory of the Pacific Islands, were aggregated. The annual incidence rate for the unit as a whole from 1979 to 1983 was 114/100,000. This is a decline from the period from 1973 to 1978 (232/100,000), which may in fact reflect a general decline in morbidity reporting that came with decentralization in mid-1978. The individual units show significant differences based on the 1982-83 statistics (Table 3). The 2/100,000 in the Federated States of Micronesia undoubtedly represents underreporting. Palau reported no cases, but the residents of Palau have long prided themselves on the fact that their fish are safe to eat. Earlier reports (Hiyama, 1943; Pacific Islands Territory High Commissioner, 1961; Banner and Helfrich, 1964) indicated that in the Caroline Island chain (with Palau to the west and the Federated States of Micronesia arching to the east), toxic fish were found in the eastern islands but absent in the west. The incidence for the Marshalls, 282/100,000 annually, was the highest reported from the former Trust Territory and may still represent significant underreporting. Ciguatera incidence in the Marianas archipelago, including Guam, is apparently increasing (Tables 1, 3).

Incidence is also low on the independent phosphate rich island of Nauru, 15/100,000. The low incidence may reflect poor reporting or reduced fishing effort. The Nauruans have one of the highest per capita incomes in the world and are highly dependent on imports. In Kiribati, ciguatera is a significant and an apparently increasing problem, with an annual incidence of 462/100,000, from 1979 to 1983. Kiribati is also one of the newly independent states of the Pacific facing the greatest development challenges. It is an atoll nation with limited land area, rapidly growing population (densities in urban Tarawa reach 1,137 km^2), and there are very limited natural resources.

Less information on ciguatera is available from the Melanesian realm. By and large, these are larger, less developed islands with both interior and coastal populations. In general, they are less dependent on the resources of the ocean. Papua New Guinea, with 61 percent of the region's population, has been excluded from the regional computations. The population of Papua New Guinea is widely distributed in the Highlands and other interior locations. Many are not dependent on marine resources and morbidity reporting is very

poor. Inclusion of Papua New Guinea would only magnify underreporting in the region as a whole. Reports from the Solomon Islands are almost as poor. The reported incidence of 2/100,000 undoubtedly reflects underreporting, but it is difficult to get an accurate picture of the situation there. Somewhat better information exists for both Vanuatu and New Caledonia. In Vanuatu where morbidity reporting is poor and the annual reported incidence was only 19/100,000, Bagnis (1977) estimated an incidence rate of 130/100,000. More recently, Guillo (1984) estimated that there were 600 cases a year (512/100,000), thus Vanuatu would be experiencing the mean for the Pacific. The Melanesian island of New Caledonia is one of the places in the Pacific where there has been an apparent decline in ciguatera incidence over the last several years. Known locally as "la gratte" for the intense itching that is often one of its symptoms, ciguatera incidence was high from 1975 to 1978 (Table 1) and has declined since then. While reported ciguatera incidence is low in Fiji, researchers at the University of the South Pacific Institute of Marine Resources, led by U. Raj, have been monitoring the situation in the Fiji group. More than 17 species were confirmed to be toxic either experimentally or from medical records (Raj et al., 1982). Discussions with both researchers at the Institute of Marine Resources and medical staff indicate that ciguatera is indeed a problem and may be on the increase. Researchers (Yasumoto et al., 1984) found two different toxins in the roe and another in the viscera of *Etelis carbunculus*, a deepwater snapper from Lauthala Island. The chromatographic properties of the toxins were different than those of ciguatoxin.

Hawaii continues to experience ciguatera outbreaks. Between 1975 and 1981 there were 81 outbreaks involving 203 individuals, giving an annual rate of 3/100,000 (Anderson et al., 1983). The authors estimated that this represented about 10 percent of the actual intoxications. In 1982, incidence was low with only 18 cases reported; 51 were reported in 1983 and 80 in 1984. It is tempting to speculate that the increase in 1984 was due to damage caused by hurricane Ewa which hit Hawaiian shores in November 1983. While the evidence is not conclusive, at least 50 intoxications have been reported from Kauai during the first 4 months of 1985. Kauai was the hardest hit of all the islands in the Hawaiian chain. In Australia, too, the ciguatera problem is apparently escalating. For decades, episodes of ciguatera poisoning have been reported sporadically from the great Barrier Reef of Northern Queensland. Reef species have usually been responsible, including the commercially important *Plectropoma maculatum*. However, since 1976, fish from Southern Queensland, in the vicinity of Hervey Bay, have been incriminated. It is the commercially important pelagic *Scomberomorus commersoni* or Spanish mackerel that is the main species responsible. Lewis and Endean (1983) have isolated the ciguatoxin-like substance from Southern Queensland *S. commersoni*.

The information that we have on the distribution of ciguatera in the Pacific basin comes from incomplete morbidity reporting and a few circumscribed, intensive investigations of the distribution of both *G. toxicus* and toxic fish. The information is far from complete and the fact that few cases are reported from the large less developed Melanesian island groups in the western Pacific, Papua New Guinea, the Solomons, and Vanuatu must be viewed with the realization that morbidity reporting for all causes from these groups is very poor. Despite this, it does seem that ciguatera is a more serious and escalating health problem in the eastern Pacific, notably French Polynesia, and in several of the more isolated island groups of the north central Pacific, Tuvalu, Tokelau, Kiribati, and the Marshalls. The anomalies in the Pacific distribution of ciguatera also deserve attention, e.g., the Cook Islands. One fruitful research frontier that may provide clues to help to unravel the ciguatera mystery is comparative analysis in the Pacific and Caribbean. It is disturbing that, in the Pacific, some of the most vulnerable island groups are the ones experiencing an elevated incidence of ciguatera.

Current Impact in the Pacific Region

While ciguatera remains a problem for islanders living in subsistence communities in the Pacific, its impact is exacerbated by the changing nature of Pacific residence, life-style, fishing, and marketing. Excluding the large, less developed islands of Melanesia, where ciguatera may generally be less of a problem, many Pacific nations have from 40 to 100 percent of their population living in the main urban center. Wage labor, often for the government, is common and individuals are moving farther and farther away from subsistence life-styles and modern aspirations are replacing traditional ones. At the same time, Pacific populations are growing, migration to the urban center continues, reef and shore modification projects are undertaken, particularly near urban centers, and there is an increased likelihood for the creation of ciguatoxic biotopes in the vicinity of the highest population densitites. Populations are becoming increasingly dependent on the purchase of fresh fish in urban markets and fish export is being encouraged. I have written at length about how island dwellers have adapted to the existence of ciguatera, beliefs about etiology and strategies for detection, prophylaxis, and cure (Lewis, 1981, 1983, 1984b). Traditional strategies, relatively effective in subsistence situations, become less so in urban areas, and when export from the point of capture is commonplace.

Morbidity statistics underline the importance of ciguatera as a public health problem in the Pacific. Except in the most highly toxic locations, it is exceedingly difficult to assess the impact of ciguatera on community nutritional status. Disease patterns are now emerging in the Pacific that mirror those of the developed world with elevated rates of diabetes, hypertension, and cardiovascular disease. Furthermore, there may be a genetic predisposition to these conditions, at least in Polynesian populations (Prior, 1981). Dietary patterns that rely on western processed foods exacerbate the health risks in these vulnerable populations, and ciguatera may

increase the tendency to rely more heavily on imported foods, both canned fish and meat and other refined, high-fat and carbohydrate foods. While it is still difficult to unravel the many factors involved, it may be easier to determine the impact of ciguatera on small-scale fisheries development.

By and large, local small-scale commercial fisheries are very poorly developed in the Pacific. In the region as a whole, the pelagic catch by Asian fleets is many times greater than the local fishery. With the establishment of the 200-mile Exclusive Economic Zones, island nations have begun, not without problems, to participate in this fishery, primarily through licensing agreements and joint venture projects. This participation is obviously important for economic development, but is does not obviate the need for the development of local small-scale commercial fisheries. There are many reasons to encourage subsistence and artisanal commercial fisheries development and the consumption of fresh fish, from benefits to the local economy, to a reduced dependence on imported food.

Accurate, comparable statistics on local catch, both subsistence and small-scale commercial fisheries, are very difficult to obtain. In the late 1970's, for example, in American Samoa, based on fish catch assessment surveys, local production was estimated to be 316,338 kg/year. Some other annual catches include: Cook Islands 606,654 kg; Fiji 5,030,362 kg; French Polynesia 2,885,667 kg; Tonga 1,039,089 kg, and Western Samoa 1,307,955 kg (this last includes subsistence catch only) (Lewis, 1981). Kent (1980), citing 1977 FAO data, gives local catch from the region as a whole as 82,696 t. This is approximately one-tenth of 1 percent of the total global catch and, while proportional to the region's population, is very low considering access to fish stocks. This figure includes local pelagic catch but not subsistence catch. Salvat (1980) estimated that the reef and lagoon catch for the region as a whole to be as high as 100,000 t. Nonetheless, in many Pacific locations as much as 90 percent of the fish eaten comes out of a can. It is difficult to generalize, but while overfishing and reef depletion have occurred where population densities are high, in the region as a whole, more fish could be taken on a sustained yield basis.

Ciguatera has been only one factor, and probably a relatively minor one, in fisheries development in the Pacific. With the changing nature of fish marketing and export, however, cinguatera or the specter of ciguatuera may have increasing impact. Many factors have contributed to change in traditional dietary patterns, to different agricultural and fishing practices, and to a dependence on imported food. One result has been that canned mackerel and sardines are often considerably less expensive than fresh fish. In 1977 the price of the least expensive fresh fish in the Cook Islands, French Polynesia, and Fiji ranged from (Australian) $1.34 to 1.50/kg. The price of canned mackerel (424 g), all of which is edible, was 43-48 Australian cents (A$ 1.00 = US$ 1.14). It is also widely distributed, relatively nutritious, and keeps without refrigeration. Furthermore, though the majority of Pacific islanders would respond that they prefer fresh fish, canned fish is a popular, easy, and relatively inexpensive protein source.

What evidence is there from the Pacific to suggest that ciguatera has affected or may affect the market for fresh fish? Urban consumers have a more generalized fish avoidance, e.g. "all the red fish" or "all the very big fish," and highly suspect species, *Lutjanus bohar*, *Lycodontis* (*Gymnothorax*) *javanicus*, or new or unfamiliar species, than individuals in subsistence situations (Lewis, 1981). This makes sense, as the urban consumer is commonly further removed from knowledge of the marine realm, may have no knowledge of who caught the fish, most likely will not know where it was caught, and, if it is filleted, may or may not know what species it is. Despite this, the ultimate responsibility for choosing a "safe" fish usually rests with the purchaser. A publicized outbreak of ciguatera associated with fish purchased from the urban market will have an adverse effect on the future marketability of that species, as it did with a catch of toxic *L. bohar* sold in the municipal market in Apia, Western Samoa. A gift to a church group of a large barracuda from an Asian longliner offloading at one of the canneries in American Samoa had a similar effect. In a follow-up study (Lewis, 1981), all those who had been poisoned said that they would avoid barracuda, and the church group voted not to serve it again.

Of all the intoxications reported in Tahiti in 1976, 52 percent were from fish caught outside of Tahiti and 18 percent were of unknown origin. Of those caught outside of Tahiti, 73 percent were caught in the Tuamotus. The population of French Polynesia is concentrated in the Papeete urban zone and the local reef resource is depleted. There is a ready market for reef fish in Papeete. Fish from several of the atolls in the Tuamotus to the north find their way to the urban market. Inhabitants on one atoll, no longer exporting fish, blamed an outbreak of ciguatera in the early 1970's for the cessation of their export. A change in interisland schooner schedules may have been equally responsible. As the statistics indicate, the fish are sometimes toxic.

Ciguatoxicity is not the only potential health problem associated with the consumption of these fish. They may be 6 days old and are often poorly iced. While the fish are not in prime condition, given the shortage of local reef fish and food preferences, they sell. This is probably the same reason that potentially ciguatoxic fish still have a market. Although one assumes that at some point if intoxications become more common or more severe, it will affect the marketability of these fish.

The Northern Line Islands of Kiribati, three equatorial islands 1,800 km south of Hawaii, loom large in that government's development plans. When there is regular airline service from Christmas Island to Honolulu, reef fish as well as spiny lobster are exported to Hawaii. Ciguatera has been a problem in the past and some species, primarily high-level carnivores, are toxic at the present time.

The potential for ciguatoxic biotopes may increase with proposed resettlement schemes. Ciguatera could also adversely effect the government's attempts to establish a sport-fishing-

oriented tourist trade. Until recently, the offshore bottom fishery (100-300 m) was poorly developed in the Pacific. It is receiving increasing attention, but some of the species caught can be ciguatoxic (Crossland, 1980).

Individuals in institutions may be at greater risk of intoxication. Large fish may be relatively inexpensive, perhaps because they are suspect, and hence find their way to institutional kitchens. In both Vanuatu and Fiji, inmates in the local jail were poisoned in the late 1970's. In 1980 in Kiribati, 54 students at the government boarding school were poisoned after a meal of barracuda (Marriott and Dalley, 1980). Large feasts are an important component of social interaction in the Pacific. The American Samoan example cited previously is only one where a single large fish prepared for a feast resulted in a common source outbreak.

Isolated populations, not only indigenous populations on outer atolls, but the crew aboard vessels or military personnel at isolated outposts can also be at risk. In 1982, 13 U.S. Navy personnel on Midway were evacuated to Hawaii after consuming a toxic *Seriola dumerili*.

Ciguatera can be a threat to the hotel and restaurant business in particular and tourism in general. The results of intoxication may include loss of business for the individual establishment and potentially for a circumscribed locale if the problem is severe enough. In the United States there is an added risk, given the litigiousness of our population.

The difficulty of assessing, except in the most highly toxic locations, the impact of ciguatera on fisheries and fisheries development has been stressed. In some parts of the Pacific, ciguatera may have been used as a convenient excuse for the lack of development. The threat of ciguatera may be as damaging as the actual incidence of intoxications. Scientific interest in the phenomenon has increased in recent years. In addition to articles in research publications, it has increasingly been the subject of letters to the editor, editorials, and articles in clinical journals in the United States, Canada, the United Kingdom, Australia, France, and the Pacific islands region. Many of these articles have alerted physicians in the United States or Europe to the fact that a patient returning from a tropical vacation with a perplexing set of symptoms may in fact be suffering from ciguatera.

Ciguatera has also become a subject of interest in the popular press. In an article in the *Australian Financial Review* (1984), the author commented that ciguatera was worrying both medical authorities and tourist promoters throughout the South Pacific. Even a few cases of ciguatera can have a drastic effect on the use of reef resources and fish avoidance can have an adverse economic impact on small-scale fisheries. In Hawaii, there is a generally increasing awareness of the problem, and interest in the origins of the red snapper that is commonly sold in large supermarket chains. It is carefully identified as New Zealand red snapper, undoubtedly because of this.

The research community, clinicians, fisheries officers, and the general public are becoming more aware of the existence of ciguatera. This can obviously have many positive effects from increased research funding, to correct diagnosis and appropriate detection and control strategies. However, care must be taken that the magnitude of the problem not be exaggerated. Tatnall et al. (1980) present a case where a West Indian was poisoned after consuming dried fish brought back from Antigua. They suggest that control in Britain rests on strong discouragement by such bodies as the Fishmongers' Company of commercial importation of moray eels and potentially ciguatoxic fish, such as amberjack and barracuda. Wholesalers do have a responsibility that should be self-evident. In the United States the potential for legal action elevates the stakes. The radioimmunoassay testing of amberjack (kahala) in Hawaii was successful; there were no intoxications from commercial purchased amberjack during the test period, but it was prohibitively expensive and also resulted in false positives. What is needed, of course, is a more complete understanding of the etiology, ecology, and epidemiology of ciguatera and a simple, inexpensive test to determine toxicity. Until then, we will have to rely on correct species identification, care to avoid both internal sale and export of toxic species, communication, and cooperation among fishermen, wholesalers, retailers, and the scientific and medical community.

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Identification and Epidemiological Analysis of Ciguatera Cases in Puerto Rico

G. ESCALONA de MOTTA, J. F. FELIU, and A. IZQUIERDO

ABSTRACT—A survey of the emergency room records of 10 hospitals in five areas of Puerto Rico from 1980 to 1982 disclosed 122 apparent ciguatera cases involving 212 individuals. Assuming that these records represented 10-15 percent of the total number of cases during this period, an estimate of 8-11 cases per 10,000 residents per year was calculated. The sample obtained was separated in two groups based on the clinical symptoms present. The frequency distribution of several demographic indicators, episode descriptors, and clinical symptoms was analyzed. The most frequent toxic fish in coastal towns was barracuda, Sphyraena barracuda, *while hogfish (Labridae) and grouper,* Epinephelus *spp., were the most toxic in the metropolitan area. Toxic episodes occurred during all months of the study period. Most episodes involved fish eaten at the evening meal and the first symptoms, all gastrointestinal, appeared 1-7 hours later. The type and frequency of occurrence of the clinical symptoms agreed well with those reported in other Caribbean studies.*

Introduction

Ciguatera is a type of food poisoning caused by ingesting any one of a wide spectrum of tropical fish. The disease is endemic in parts of the tropical Pacific and the Caribbean. The current hypothesis concerning the origin of ciguatoxin is that specific dinoflagellates living in association with macroalgae produce toxins that are accumulated in the tissue of fish through the food web of the coral reef. Humans acquire the disease by eating the flesh of these toxic fish.

Toxic fish have a normal appearance, taste, and smell, and the toxins they contain are not inactivated by cooking or refrigeration. There are no specific laboratory tests for the disease, and diagnosis depends on the clinical signs and symptoms presented by the patients. These usually begin with gastrointestinal (GI) disturbances which are followed by neurological and cardiovascular symptoms. Sensory disturbances such as paresthesias of the perioral region and the distal extremities, generalized pruritus and abnormal temperature sensations are considered distinctive features of the disease (Lawrence et al., 1980).

Although the disease is characterized by a low mortality rate, it is occasionally fatal and represents a major cause of morbidity in areas where it is endemic (Bagnis et al., 1979; McMillan et al., 1980). Sometimes the neurological disturbances are prolonged, resulting in alterations in the patient's normal life patterns. In addition, the existence of this disease has important economic implications for the fishing industry in tropical areas.

In view of the medical, social, and economic importance of this disease, this study was initiated to gather data on the occurrence and clinical manifestations of ciguatera cases in Puerto Rico. The results of this study complement available epidemiological data giving a more complete picture of ciguatera in the Caribbean.

Methods

Sample Selection

Information concerning ciguatera cases in five coastal regions of Puerto Rico during 1980, 1981, and 1982 was obtained through a survey of the emergency room records of 10 major public and private hospitals. In addition, the records of the Puerto Rico Poison Control Center, as well as patients and private physicians, were consulted to supplement this information. The localities studied and the approximate size of the population served by these hospitals are shown in Figure 1. Cases in which the patient's major complaints were acute GI disturbances, or whose final diagnosis was either ciguatera fish poisoning or food poisoning, were identified by consulting the records of the hospital emergency rooms. Only those patients with GI symptoms within 24 hours of eating fish were selected for further study.

A questionnaire was designed to organize the data obtained from the hospital records and interviewees. It contained five demographic indicators, 10 episode descriptors, and questions about the onset, duration, and intensity of four GI and 17 neurological symptoms. Using this form we were able to identify 122 apparent ciguatera episodes involving a total of 212 individuals. All individuals eating from the same fish were considered as belonging to the same episode.

Data Analysis

Files were created for each identified individual using the missing data code whenever information was not available. To identify more closely those cases presenting what is generally considered

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the characteristic symptomatology of the disease, the sample was divided into two groups. Group A consisted of those cases where at least one individual per episode, in addition to the GI symptoms, showed any two of the following neurological alterations: Malaise, pain, paresthesias, temperature inversion, metallic taste, or pruritus. The cases not meeting these selection criteria formed group B. The frequency distribution in both groups of all the variables studied was determined using the Statistical Package for the Social Sciences (SPSS) computer program. Relationships among selected items were also established using cross-tabulations.

Results

Demographic Characteristics

In both groups, the most affected persons were adults, nearly half of whom were in the 20-30 years range (Table 1). Sex ratios were also similar in both groups.

Episode Characteristics

The type of fish eaten was reported by 68 individuals in each of the two groups representing 85 percent of group A and 52 percent of group B (Fig. 2). Hogfish (Labridae) (28 percent) and grouper, *Epinephelus* spp. (26 percent), were the fish most frequently reported in group A, while in group B, barracuda, *Sphyraena barracuda* (46 percent), was the most frequently reported species. Although some of the fish were caught by the victims themselves, most of the ciguatoxic fish were purchased in fish markets or eaten at restaurants, particularly in the metropolitan area.

Distribution of the cases among the five surveyed areas is shown in Figure 3. Most of the cases included in group A were from the metropolitan area (43 percent) but only 3 percent of those in group B were from this locality. The rest of the cases in the latter group were equally distributed among the other four areas: Fajardo (27 percent), Humacao (25 percent), Ponce (23 percent), and Mayaguez (22 percent). No toxic barracuda were involved in the episodes reported in the metropolitan area (Table

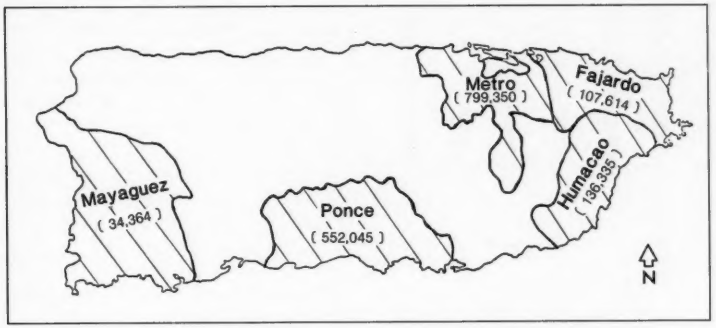

Figure 1.—Areas included in the epidemiological survey of ciguatera in Puerto Rico. Areas outlined are labelled as named in the study, and numbers in parentheses indicate the approximate size of the population served by the medical facilities surveyed. Data were obtained from the Statistics Division, Health Department, Commonwealth of Puerto Rico.

Table 1.—Age and sex distribution of apparent ciguatera cases in Puerto Rico from 1980 to 1982, grouped by symptom pattern.

Item	Group A	Group B
Age (years)	(*n* = 76)	(*n* = 132)
≥19	10 (13%)	31 (23%)
20-39	39 (13%)	53 (40%)
40-59	20 (26%)	35 (27%)
≤60	7 (9%)	13 (10%)
Sex	(*n* = 80)	(*n* = 132)
Male	45 (56%)	75 (57%)
Female	35 (44%)	57 (43%)

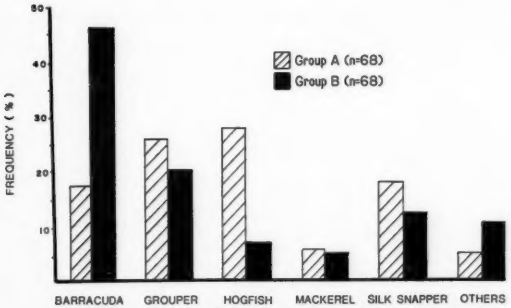

Figure 2.—Types of fish involved in the ciguatera cases identified. Frequency is indicated as percent of the cases (n) reporting the common name of the fish consumed.

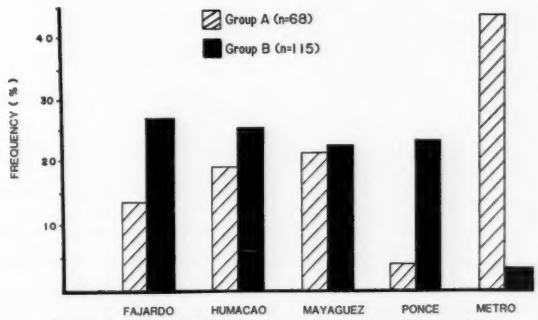

Figure 3.—Distribution of the ciguatera cases identified among the five areas surveyed. Frequency indicated as percent of the cases (n) reporting the locality where toxic fish was consumed.

2). Here, hogfish, grouper, and silk snapper, *Lutjanus vivanus*, were the reported toxic fish. Barracuda were the most frequent toxic fish in the areas that are important fishing centers, such as Fajardo and Humacao.

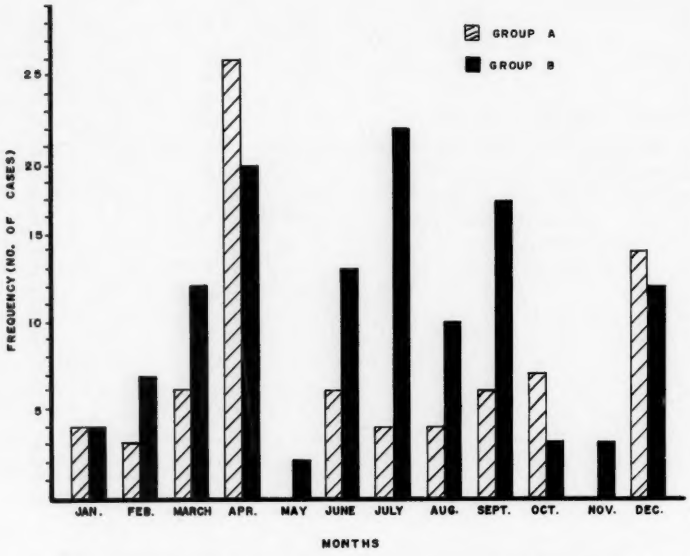

Figure 4.—Distribution of the total number of ciguatera cases identified in the study by month of occurrence. Size and characteristics of groups A and B are described in the text.

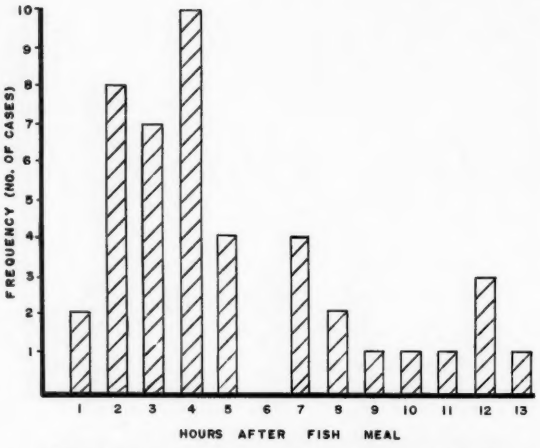

Figure 5.—Onset of first ciguatera symptoms. The time elapsed after fish consumption for the appearance of GI symptoms is indicated in the abscissa. Data obtained from cases in group B reporting fish consumption at evening meal.

Toxic episodes occurred during all months of the study period. Peaks were observed in both groups in April and also during summertime in group B (Fig. 4). In group B, 60 percent of the episodes resulted from eating fish at the evening meal (6-8 p.m.) and 21 percent from fish eaten at lunch time (12-2 p.m.). Those persons that became sick from eating the fish at the evening meal were selected for information regarding onset of symptoms. Among these, the first symptoms appeared 1-7 hours after ingestion of fish (Fig. 5).

Symptomatology

The type and frequency of GI and neurological symptoms reported by patients is shown in Table 3. As expected from the original sample selection procedure, GI symptoms occurred with similar frequencies in groups A and B, with diarrhea being the most common disturbance. From two to four of these symptoms appeared concurrently in an individual in over 80 percent of the samples. Interestingly, in group A, selected specifically by the presence of a given set of neurological symptoms, all four GI symptoms appeared together in 53 percent of the patients. A few individuals (7 percent) did not report any GI disturbance but were added to this analysis since they belonged to episodes meeting the criteria for inclusion.

Among the neurological symptoms studied, the most frequent of those used as selection criteria for group A was malaise (65 percent) followed by arthralgia (60 percent), and myalgia (56 percent). In this group, characteristic sensory disturbances such as paresthesia

Table 2.—Frequency distribution of fish involved in the toxic episodes studied in Puerto Rico by area surveyed.

	Number of cases					
Type of fish[1]	Fajardo	Humacao	Mayaguez	Ponce	Metro	Total
Barracuda	14	13	10	6	0	43
Grouper	1	12	11	0	6	30
Hogfish	5	0	0	1	13	19
Silk snapper	3	0	7	0	7	17
Others	4	2	3	1	1	11
Total	27	27	31	8	27	120

[1]Fish common names are those reported by patients to emergency room physicians or interviewers.

of the fingers and temperature inversion were present in 54 and 48 percent of the patients, respectively. However, metallic taste, recognized as another peculiar feature of the syndrome, was apparent in only 25 percent of the patients included in group A. Within group B, dizziness appeared in 32 percent of the cases, but no other neurological symptom exhibited a frequency above 13 percent.

It was not possible to analyze the frequency of occurrence of cardiovascular symptoms in our sample since, unfortunately, adequate reports of vital signs determinations were absent in over 80 percent of the cases identified.

Discussion

The existence of ciguatera poisoning in the Caribbean is well documented in the literature but the epidemiological data available to date is fragmentary and limited (Ragelis, 1984). Thus, estimates about the incidence of the problem in this area are questionable. The data on the incidence of poisoning episodes in Puerto Rico obtained in this study may also be incomplete and biased towards the more acute cases due to our use of hospital records as the main source of information and the fact that not all the hospitals in each area were surveyed.

Assuming that the cases identified represented only 10-15 percent of the actual cases occurring annually in the areas studied, one may estimate this incidence as 8-11 cases per 10,000 residents. This estimate is based on similar calculations made for the population of the U.S. Virgin Islands (McMillan et al., 1980). As indicated in Figure 1, residents in the areas studied represent about half of the total island population. This calculation was made using only cases reported from June 1981 to December 1982, since those were over 90 percent of the total sample obtained. The large number of cases identified during that period may be related to an increased awareness about the disease after press reports on the death of a ciguatera victim (Ghigliotty, 1981). This also resulted in a government ban on the sale of barracuda and a few other possibly ciguatoxic fishes. In this study, age and sex did not appear to influence occurrence and type of symptoms experienced in a toxic episode.

Information concerning types of fish eaten showed that barracuda was the most frequent toxic fish but only in the coastal towns, where it is primarily consumed by fishermen and their families. This, undoubtedly, reflects the fact that this fish may not be sold commercially in Puerto Rico. Similarly, Lawrence et al. (1980) reported that barracuda is frequently a toxic fish in Miami where its sale is also prohibited.

Our data did not show any seasonality of the disease, as ciguatera episodes were identified in every month of the year during the study period. The peaks observed in April and during summertime may reflect an increase in fish consumption rather than peaks in fish toxicity. In particular, the peak in April is probably related to more use of fish in the diet during Lent.

The diagnosis of ciguatera cases depends on the patient's clinical presentation, since there is a poor understanding of the pathophysiology of this disease. This clinical picture, apparent from our study as well as from other Caribbean studies (Table 4), includes early GI symptoms, appearing a few hours after eating toxic fish. The concurrent appearance of all four gastric disturbances in over 50 percent of the cases selected for presence of certain neurological symptoms (group A) documents the importance of these symptoms as a manifestation of the disease. In group B, which included those cases not reporting together the set of symptoms considered characteristic of the disease (Table 4), no obvious association of GI and neurological symptoms was observed. Thus, concurrent appearance of GI, neurological, and, possibly, cardiovascular symptoms appearing at a later time still seems the best criterion for an initial diagnosis of ciguatera.

Table 3.—Frequency distribution of symptoms in all cases studied, grouped by symptom pattern.

Symptom	Percent of patients with symptom	
	Group A (n = 80)	Group B (n = 132)
Gastrointestinal		
Nausea	69	70
Vomiting	69	42
Diarrhea	83	77
Abdominal pain	74	62
Neurological		
Malaise	65	13
Arthralgia	60	9
Myalgia	56	6
Paresthesia		
Fingers	54	10
Perioral	38	3
Extremities	36	13
Temperature inversion	48	2
Pruritus	45	5
Metallic taste	25	2
Headache	39	11
Dizziness	33	32
Respiratory disturbances	18	2
Visual disturbances		
Photophobia	11	2
Others (diplopia, etc.)	16	4
Irritability	13	0
Toothache	11	1
Unconsciousness	4	1

Table 4.—Frequency (%) of various ciguatera symptoms reported in Caribbean studies.

Symptom	Barkin (1974)	Lawrence et al. (1980)	Morris et al. (1982)	Engleberg et al. (1983)	This study (Group A)
Gastrointestinal					
Diarrhea	100	76	91	81	83
Vomiting	100	68	70	40	69
Abdominal pain	88	*	39	64	74
Neurological					
Headache	13	47	33	45	39
Pruritus	50	48	58	66	45
Malaise	*	30	70	*	65
Myalgia	75	86	30	34	56
Arthralgia	88	*	52	34	60
Paresthesia, fingers	4	*	40	*	54
Paresthesia, extremities	*	71	33	*	36
Perioral paresthesias	29	54	36	38	38
Temperature inversion	*	*	36	23	48
Metallic taste	*	*	27	*	25
Dizziness	*	*	21	*	33

*Not reported.

Neurological alterations seen more frequently in the selected sample A and appearing 12-24 hours after fish ingestion were malaise or general weakness, bone and muscle pain, distal paresthesias, and temperature inversion. Dizziness appeared almost equally frequent in both groups, suggesting that it could be a side effect of the intense GI disturbances reported by all patients in the sample. Close agreement between ciguatera studies in the Caribbean (Table 4) suggests that our conclusions concerning the proper diagnosis of this disease may be applied to most cases occurring in this region.

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The Chemical Nature of Scaritoxin

YONG-GOE JOH and PAUL J. SCHEUER

Introduction

One of the many questions associated with ciguatera research has been that of the multiple nature of the toxins that give rise to ciguatera symptoms in man. The lipophilic ciguatoxin (Scheuer et al., 1967) and the hydrophilic maitotoxin (Yasumoto et al., 1976) are distinct and reasonably well characterized molecules, though their specific contributions to the ciguatera syndrome have not been assessed.

Bagnis et al. (1974) conducted an epidemiological survey of ciguatera intoxication in the Gambier islands and observed that the most frequently implicated fish were parrotfish (Scaridae). Afflicted persons would, in addition to the conventional immediate ciguatera symptoms, suffer significantly from delayed (by 5-10 days) and prolonged (1 or more months) episodes of disturbed equilibrium, locomotor difficulties, and kinetic tremors.

An obvious explanation is the presence in parrotfish of a new toxin called scaritoxin, or of two distinct toxic entities (Chungue et al., 1977a, b). From the flesh of *Scarus gibbus*, Chungue et al. (1977a, b) successfully separated two toxins by chromatography on DEAE cellulose. A toxin eluted with chloroform and designated SG-1 evoked sluggishness and severe hind limb paralysis in mice, different from typical ciguatoxin symptoms. A second toxin eluted with chloroform-methanol (1:1) and designated SG-2 produced conventional ciguatoxin symptoms in mice, such as diarrhea, lachrymation, salivation, and respiratory difficulties.

Both toxin entities were further purified on Sephadex LH-20. SG-1 was a yellowish oil with an LD_{50} of 0.03 mg/kg. The polar toxin SG-2 was compared chromatographically with moray eel ciguatoxin and was found to be indistinguishable. Both toxins, SG-1 and SG-2, responded negatively to reaction with iodine and to Dragendorff and Jaffé reagents. A chromatographic estimate of the molecular weight of the new, less polar SG-1 toxin was approximately 800 daltons.

Chungue (1977) in a detailed investigation substantiated these results for six additional species of parrotfish. She concluded that scaritoxin is a ciguatera-causing toxin that is characteristic of the Scaridae.

In a more recent study, Nukina et al. (1984) showed that ciguatoxin isolated from moray eel, *Lycodontis javanicus* (≡ *Gymnothorax*), viscera can be separated on basic alumina into two entities, differing in polarity but indistinguishable in symptoms or in LD_{50} (i.p. mice). The two toxins show only minor differences in their high field ^1H NMF spectra. It was further shown that the two toxins are interconvertible. It occurred to us that these two chromatographic forms of ciguatoxin might be identical with the scaritoxin-ciguatoxin pair recognized in the flesh of parrotfish by Chungue (1977). We had an opportunity to collect parrotfish, *S. sordidus*, on Tarawa atoll, Republic of Kiribati, and thus subject this hypothesis to an experimental test.

ABSTRACT—Two toxins were isolated from Scarus sordidus *(Family Scaridae) collected at Tarawa atoll, Republic of Kiribati. Both toxins were present in the flesh and viscera, but in different ratios. They correspond to the previously described scaritoxin and ciguatoxin. The toxins can be interconverted and evoke parallel symptoms in mice. Scaritoxin is probably identical with less polar ciguatoxin, but lack of material prevented unequivocal proof by spectral comparison.*

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Materials and Methods

Fish were collected 19-27 May 1983 at Taborio, Betio, Bikenibeu, and Teaoraereke (Fig. 1). Small-scale extraction and testing showed that only *S. sordidus* caught at Bikenibeu were toxic. Flesh (5.34 kg) and viscera (0.876 kg) were worked up separately.

Fish flesh was mixed with acetone and reduced to a brei in a 1-gallon Waring Blendor[1]. The mixture was transferred to a 4 liter Erlenmeyer flask and allowed to stand in excess acetone for 2 days, then with fresh acetone for another 4 days. The combined acetone extracts were concentrated to an aqueous suspension, which was treated twice with equal volumes of hexane. The hexane layer was back-washed with 3× 100 ml of methanol/water, 8:2. The washings were added to the aqueous suspension, and the combined aqueous material was extracted three times with equal volumes of ethyl acetate. Evaporation of ethyl acetate resulted in crude toxin.

Crude toxin was dissolved in a minimum amount of chloroform and applied to the top of a column packed with Silicar (200-425 mesh, Mallinckrodt, 28 g/g sample). Successive elution with chloroform (10 ml/g silica), chloroform/

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

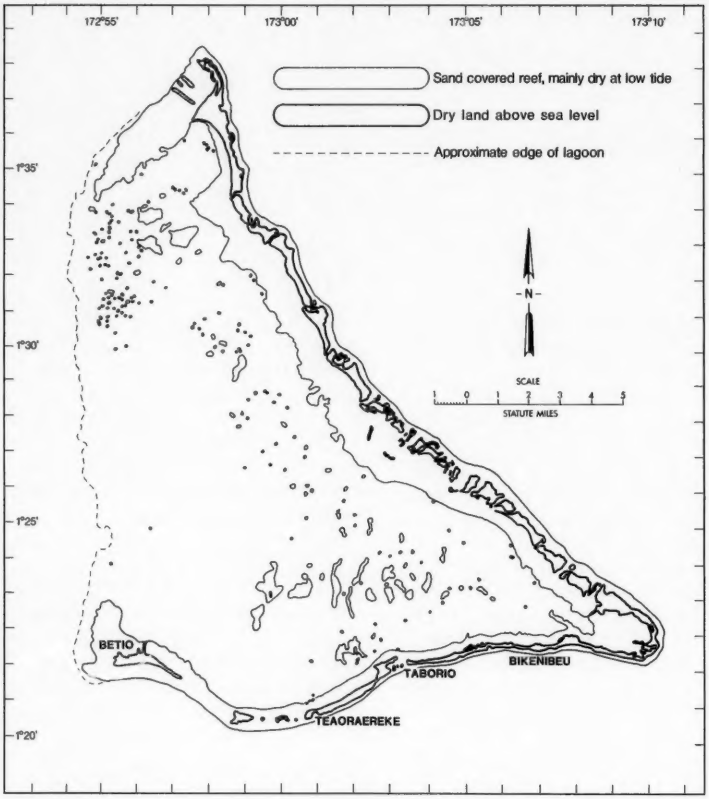

Figure 1.—Tarawa atoll, Republic of Kiribati.

methanol (9:1, 14 ml/g silica), chloroform/methanol (1:1, 10 ml/g silica), and methanol (10 ml/g silica) separated the toxin. All fractions were evaporated to dryness immediately after elution.

The chloroform/methanol fraction (9:1) was further purified on a DEAE celulose column (Cellex D, acetate form, BioRad Laboratories, 9.3 g/g sample), which was prepared as previously described (Rouser et al., 1963). Eluting solvents were chloroform (60 ml/g adsorbent), chloroform/methanol (1:1, 90 ml/g adsorbent), and methanol (60 ml/g adsorbent). Guaiazulene (a blue pigment, Aldrich Chemical Co.) was used to determine the bed volume and to detect imperfections in the packing.

An alumina (activity grade I, Woelm) column was prepared by suspending alumina (100 g/g sample) in chloroform. Toxic fractions were added as chloroform solutions and were eluted successively with chloroform/methanol (100:1, 9:1, 1:1), methanol, methanol/water (1:1), 375 ml/g alumina.

Alumina activity grade V was prepared according to the Brockmann scale. Grade I and V alumina columns (18.5 × 0.7 cm) were packed with 10 g alumina each and the toxins were eluted with chloroform (40 ml), chloroform/methanol (36 + 4 ml), chloroform/methanol (1:1), methanol (40 ml), and methanol/water (1:1). The eluates were concentrated and the residues were dissolved.

The toxic fractions (polar toxin, 450 ng; less polar toxin (810 ng) were further purified on a Sephadex LH 20 (Pharmacia) column (103 × 1.4 cm) by elution with chloroform/methanol (2:1). Fractions (4.9 ml) were collected every 10 minutes. Elution was monitored at 254 nm (ISCO Model UA-5).

Aluminum plates coated with silica gel 60F-254 (0.2 mm) and glass plates spread with silica gel H were used for thin-layer chromatography (TLC). Plates were developed with chloroform/methanol/water/acetic acid (90:9.5:0.3:0.2) and visualized with iodine vapor. Spots or bands were scraped off and extracted with chloroform/methanol, 4:1 for the less polar and 1:1 for the polar toxin. For bioassay the recovered TLC fractions were homogenized in 1 percent Tween 80.

For bioassay, each sample was dissolved in a known volume of methanol. Aliquots were removed from this stock solution and solvent was removed by a stream of nitrogen. Tween 80 (0.5 ml of 1 percent or 0.1 ml of 5 percent) was added and homogenized in a Vortex mixer. Swiss Webster mice (male or female, 16-22 g) were injected intraperitoneally. Death times of less than 3 hours were used to estimate toxicity as described by Tachibana (1980).

Results

Small-scale extractions and bioassay showed that *S. sordidus* was the only toxic species; *S. frenatus*, *S. scaber*, and *S. pectoralis* were nontoxic.

Yields and lethalities of extracts and chromatographic fractions are shown in Tables 1 (flesh) and 2 (viscera). The viscera proved to have a higher concentration of toxin, a phenomenon that had previously been observed in the moray eel (Yasumoto and Scheuer, 1969). Toxin could be eluted from silicic acid cleanly in chloroform/methanol (9:1). The toxin was separable into polar and less polar entities on DEAE cellulose. In *S. sordidus* flesh the polar toxin predominated, while in the viscera the reverse was so. When the chloroform/methanol (1:1) fraction from the DEAE cellulose column was rechromatographed on alumina activity grade I and

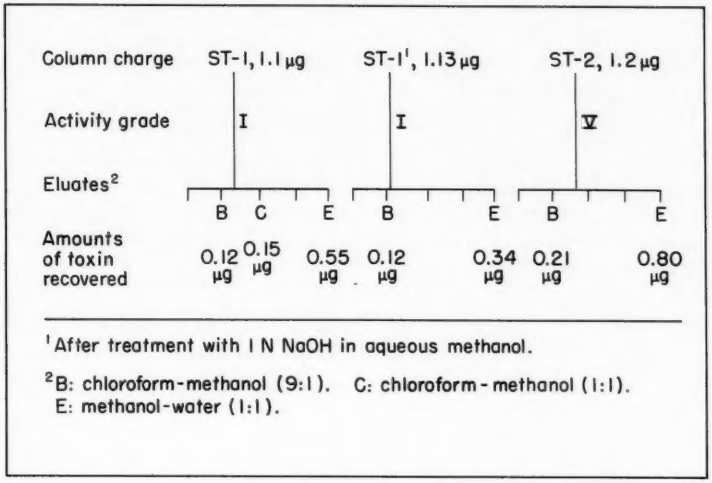

Figure 2.—Interconversion of *Scarus sordidus* flesh toxins on alumina.

Table 1.—Yields and toxicity of extracts and of chromatographic fractions of *S. sordidus* flesh (5.34 kg).

Purification state	Yield (g)	LD_{50} (mg/kg)	Total toxicity (M.U.)
Ethyl acetate	8.99		
Silicic acid			
Chloroform	3.52	Nontoxic	
Chloroform/methanol (9:1)	1.53	23.0	3,319
(1:1) (9:1)	0.63	12.5	2,520
(1:1)	1.13	Nontoxic	
Methanol	2.20	Nontoxic	
DEAE-cellulose			
Chloroform	2.74	29.0	556
Chloroform/methanol (1:1)	0.52	22.5	1,160
Methanol	0.01	Nontoxic	

Table 2.—Yields of extracts and of chromatographic fractions of *S. sordidus* viscera (0.876 kg).

Purification state	Yield (g)	LD_{50} (mg/kg)	Total toxicity (M.U.)
Ethyl acetate	13.81		
Silicic acid			
$CHCl_3$	4.46	Nontoxic	
$CHCl_3$-CH_3OH (9:1)	5.05	29	8,763
$CHCl_3$-CH_3OH (1:1)	1.48	Nontoxic	
CH_3OH	1.58	Nontoxic	
DEAE-cellulose			
$CHCl_3$	2.94	29.5	5,034
$CHCl_3$-CH_3OH	1.00	27.8	1,799
CH_3OH	0.08	Nontoxic	

Table 3.—Comparison of R_f-values of *S. sordidus* flesh toxins with PCTX (Tachibana, 1980) and scaritoxin (Chungue, 1977). Aluminum plates were coated with silica gel 60F-254 (0.2 mm); solvent system was chloroform/methanol/water/acetic acid (90:95:0.2:0.3).

ST-1	ST-2	PCTX	Scaritoxin
0.60–	0.30–	0.28–	0.78–
0.75	0.54	0.54	0.92

eluted with a gradient beginning with chloroform/MeOH (100:1) and ending with methanol/water (1:1), 95.5 percent toxicity was eluted with methanol/water (1:1). The two toxins gave rise to parallel symptoms in mice.

In analogy with the recent demonstration (Nukina et al., 1984) that ciguatoxin from moray eel viscera can be separated into two distict entities of different polarity by alimina chromatography and that the two toxins are interconvertible, we were able to show that the two *S. sordidus* toxins obtained by DEAE cellulose chromotography can also be partially interconverted. Figure 2 shows that ST-1 is partially converted to ST-2 when passed through a column of highly active (Grade I) alumina. ST-2, on the other hand, can be partially converted to ST-1 by chromatography over deactivated (Grade V, 15 percent water) alumina. When ST-1 is first treated with aqueous methanolic sodium hydroxide, then chromatographed on alumina of activity I, partial conversion to ST-2 also takes place, but the loss of material is necessarily severe. Paucity of available toxin prevented us from carrying out a full-scale experiment. Results are shown in Figure 2. The less polar toxin is designated ST-1, the polar toxin, ST-2.

On subsequent chromatography on Sephadex LH 20, ST-1 and ST-2 are eluted in parallel fractions (88-110 ml for ST-1 and 86-108 ml for ST-2), thus demonstrating the identical size of the two toxins.

To show the relationship of the two *S. sordidus* toxins to ciguatoxin (Nukina et al., 1984) and to scaritoxin (Chungue, 1977), we carried out thin layer chromatography under Chungue's conditions. Within the normal variability of TLC with time and place, and with a four-solvent developer, ST-1 and scaritoxin are very likely to be identical, as are ST-2 and ciguatoxin (Table 3). The TLC spots are egg-shaped rather than circular. Significantly, though, the R_f-values do not overlap. Amounts of toxin sufficiently large for ^1H NMF comparison are needed for unequivocal proof.

Discussion

The demonstration of two chromatographically distinct toxins in a ciguatoxic fish, while interesting by itself, raised another question concerning the precursor(s) of the toxins. Parrotfish, *S. gibbus*, feed primarily on coral. To shed light on the origin of the toxin, Yasumoto in collaboration with workers in Tahiti (Yasumoto et al., 1977) examined the gut contents and liver of *S. gibbus*. The gut, surprisingly, contained no scaritoxin, but contained ciguatoxin and maitotoxin in addition to a fast-acting acetone-soluble paralysis-causing toxin. Only ciguatoxin was isolated from the liver. Since both ciguatoxin and scaritoxin are routinely isolated from flesh, these results suggest that the parrotfish may have the ability to transform ciguatoxin into scaritoxin, yet the absence of scaritoxin in the liver was puzzling.

Our findings that toxic parrotfish from Tarawa atoll come from a narrow geographic area (Fig. 1) is consistent with the traditional ciguatera phenomenon (e.g., Withers, 1982). We were both surprised, though, that of four species examined, only *S. sordidus* proved toxic. We were able to isolate two toxins, parallel with Chungue's (1977) work on *S. gibbus*. In contrast to Yasumoto's (Yasumoto et al., 1977) results, we were able to show that both toxins are present in the flesh and in the viscera, albeit in different proportions. We observed no evidence of a third acetone-soluble and fact-acting toxin (Yasumoto et al., 1977). This is not a critical point, as coral-feeding parrotfish have many opportunities to ingest other toxins. Bagnis' (1974) original observation of the different symptomology in man following intoxication by parrotfish remains unexplained.

Acknowledgments

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Resolution of Ciguatera-Associated Toxins Using High-Performance Liquid Chromatography (HPLC)

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PAUL J. SCHEUER, and DAVID J. JOLLOW

ABSTRACT—There is little doubt that the human illness, ciguatera, results from ciguatoxin in contaminated fish. That toxins other than ciguatoxin may be present in some fish and may also be isolated from the putative ciguatoxin progenitor, Gambierdiscus toxicus, *has complicated studies in this area. A method is proposed that fractionates the toxic moieties present in crude fish or dinoflagellate extracts based on their relative polarities and provides a tentative identification of these toxins. Four distinct toxic entities have been identified by this method. Each of four cultured* G. toxicus *strains yielded a single, chromatographically identical toxin (putative maitotoxin).* Ostreopsis lenticularis *cultured cells yielded a much more polar toxin that eluted in the void volume. Extracts of ciguatoxic fish harvested from the Caribbean yielded a single toxic component that co-chromatographed with purified ciguatoxin. An aliquot of an extract from a ciguatoxic fish caught from the waters off Tahiti yielded two distinct toxic fractions: One fraction that co-migrated with purified ciguatoxin and a second less polar fraction presumed to be the interconvertible form of ciguatoxin, termed scaritoxin. The chromatographic mobilities of these toxins relative to various markers illustrates the usefulness of this method in providing a tentative identification of the toxins present in crude extracts of suspect fish or dinoflagellates.*

Introduction

The predominant and perhaps sole toxin responsible for the clinical manifestations of ciguatera is ciguatoxin (Scheuer et al., 1967). The toxin is a colorless solid with a molecular weight of 1112 (Tachibana, 1980) and has been the only ciguatera-associated toxin purified and chemically characterized. Two additional toxins, however, have been isolated from suspect fish; these have been termed maitotoxin (Yasumoto et al., 1976) and scaritoxin (Bagnis et al., 1974). The marine organism responsible for the biosynthesis of ciguatoxin and maitotoxin appears to be the dinoflagellate, *Gambierdiscus toxicus* Adachi et Fukuyo (Yasumoto, et al., 1977). Laboratory cultures of the dinoflagellate, however, have yielded a significant level of toxicity attributable to maitotoxin but little, if any, toxicity associated with the "ciguatoxin fraction."

Since research efforts in the area of ciguatoxin are dependent on obtaining a reasonable supply of ciguatoxin, several laboratories have initiated programs for culturing *G. toxicus* or other suspect dinoflagellates with the expectation of acquiring sufficient quantities of ciguatoxin. However, convincing evidence has yet to be presented that ciguatoxin can be isolated from laboratory culture systems. Instead, a number of toxic moieties have been reported (Dickey et al., 1984; Miller et al., 1984; Withers, 1984), which may or may not be identical and associated with the illness ciguatera.

To help resolve the identity of these toxins and to provide unambiguous data necessary to define them, a relatively simple technique involving high performance liquid chromatography (HPLC) was developed. The method described does not require extensive preparation of the cell-free extracts before chromatography and lends itself to a preparative procedure for purification.

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Materials and Methods

Dinoflagellate Toxin Source

All dinoflagellate cultures used in this study were clonal cultures maintained and harvested as described elsewhere (Sawyer et al., 1984; Babinchak et al., 1986). *G. toxicus* T-39 was isolated from Tern Island by Withers (1984), and cultured cells of this strain were supplied by Richard York (Hawaii Institute of Marine Biology, University of Hawaii) or John Babinchak. *G. toxicus*, CD-series, were cultured from clones isolated from the Florida Keys. All dinoflagellates were extracted with methanol:water (80:20) for a minimum of 24 hours at room temperature, filtered, dried under nitrogen, and stored as a stock solution in absolute methanol at 4°C. One additional laboratory-cultured dinoflagellate isolated from Puerto Rican waters and possessing limited toxicity (Ballantine et al., 1986; Tosteson et al., 1986) was *Ostreopsis lenticularis*, submitted by T. Tosteson (University of Puerto Rico).

Fish Toxin Source

Partially purified extracts of ciguatoxic fish were kindly supplied by Joseph McMillan (College of the Virgin Islands). The fish were identified as

kingfish, *Menticirrhus* sp., and barracuda, *Sphyraena barracuda*, and were caught off the coast of St. Thomas, U.S. Virgin Islands, and the extracts (McMillan et al., 1980) were pooled. An aliquot of a crude extract of ciguatoxic fish from the waters off Tahiti was supplied by Raymond Bagnis (Institute of Medical Research, Papeete, Tahiti) and extracted according to Pompon and Bagnis (1984). All fish extracts were dissolved in acetone and stored at 4°C.

HPLC Method and Conditions

Chromatographic fractionation of the dinoflagellate or fish components in the crude extract was accomplished using a C_8 silica-based reverse phase column (4.6×250 mm with 5 μ particle size; Altech Assoc.[1], Deerfield, Ill.) equilibrated in methanol:water (50:50) and protected with an appropriate guard column. Dupont Instruments 8800-series Gradient Controller, Gradient Pump, and UV Spectrophotometer (Du Pont Co., Wilmington, Del.) were used. All dinoflagellate and fish toxin samples were filtered and applied in 50 percent aqueous methanol. The eluant was monitored at 215 nm and absorbance recorded on a Shimadzu C-R3A Integrating Recorder (Shimadzu Corp., Kyoto, Japan). The eluant was collected in 1 minute fractions using a Gilson Model FC-80K Fractionator (Gilson Medical Electronics, Middletown, Wis.). At zero time, a 50 μl sample was injected and a linear gradient of methanol:water (50:50 to 100:0) was applied with a segment length of 25 minutes, after which absolute methanol was introduced. The flow rate was maintained at a constant 1.0 ml/minute.

HPLC Standards

To establish uniform HPLC operating conditions, 10 μl of a mixture of six standards or markers were run before and after each dinoflagellate or fish toxin sample. These standards included phenol (0.15 mg/ml), p-bromophenol (0.50 mg/ml), 1-chloro-4-nitrobenzene (0.20 mg/ml), toluene (0.25 mg/ml), precocene II (0.10 mg/ml), and naphthalene (0.10 mg/ml). The detection of these markers was monitored by absorption at 215 nm.

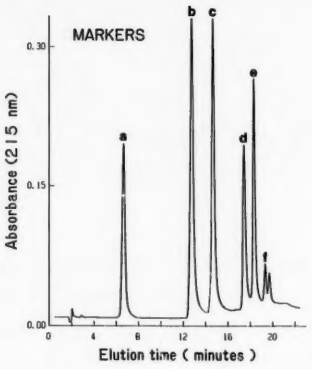

Figure 1.—U.V. profiles of selected markers used in standardizing the chromotographic conditions employed (see Materials and Methods section). Markers were phenol (a), p-bromophenol (b), 1-chloro-4-nitrobenzene (c), toluene (d), prococene II (e), and naphthalene (f).

The conditions described herein were established to optimize efficiency, selectivity, and resolution in the separation of toxicity associated with the particular test solutions. Six markers were selected based on their relative extinction coefficient at 215 nm and their relative residence time under the conditions employed. As evident from their typical chromatographic profile (Fig. 1), good separation and a distinctive elution pattern were obtained. Occasionally, an additional absorption peak at about 17.7 minutes appeared, but this peak corresponded to a contaminant in the "HPLC-Grade" water and was observed when the solvent gradient alone was run. The markers were routinely applied to the HPLC system within 3 hours before and after each toxic test sample run and over a 4-month period. The deviation in elution time over this time was minimal (Table 1) and attests to the reproducibility of the conditions employed. Because of the minimal variation in the mobility of phenol, it was used for determining the comparative elution time for the dinoflagellate and fish toxic components.

Table 1.—Retention time of markers.

Marker	Elution time (min.)[1]
Phenol	6.68 ± 0.24
p-Bromophenol	12.95 ± 0.38
1-Chloro-4-nitrobenzene	14.90 ± 0.41
Toluene	17.66 ± 0.30
Precocene II	18.52 ± 0.26
Naphthalene	19.85 ± 0.34

[1] $\bar{X}$ ± SD, n=21.

Toxicity Assay

Column fractions were placed in a stream of nitrogen until visibly dry and then transferred to a vacuum dessicator overnight. The samples were reconstituted with Tween 80 (5 percent; 0.5 ml) in phosphate-buffered saline (PBS) immediately before the assay.

Our routine bioassay for ciguatera-associated toxins was described at this Conference (Kelley et al., 1986). Each suspended fraction was administered intraperitoneally (i.p.) to two female, ICR mice (0.2 ml/mouse). For positive and negative controls, animals received either the crude extract or the Tween 80 in PBS solvent. The mice were observed for 48 hours, and their body temperature recorded at various intervals (Sawyer et al., 1984). The animal response that defined toxicity of a fraction was limited to those fractions wherein both animals died within the 48-hour test period.

Results

Separation of mixture components by C_8 columns is achieved by reverse phase partitioning between the stationary hydrophobic octasilane phase bonded to the silica gel matrix and the moving hydrophilic solvent. Residence time of a particular component on the column depends principally on its relative solubility in the stationary hydrophobic and moving hydrophilic phases. Separation of the components in the mixture in reverse phase HPLC is therefore related to their partition coefficients with the more polar substances being eluted first.

One of the first strains of *G. toxicus* placed into culture was the cloned

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

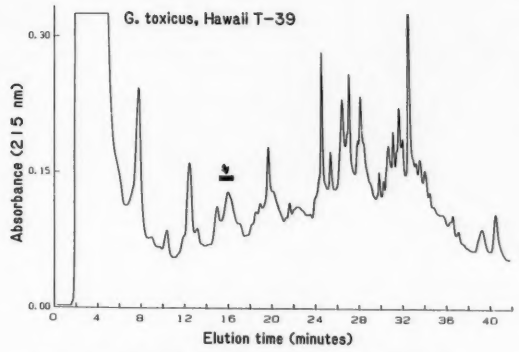

Figure 2.—Chromatographic profile of an extract of *G. toxicus*, clone T-39, isolated from Hawaiian Archipelago. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

Hawaiian strain, T-39, isolated from Tern Island. Simple methanol extraction of these cells resulted in a cell-free extract with at least a 50 percent recovery of toxicity based on a standardized LD_{50} curve (McMillan et al., 1980), using whole cells and extracts thereof. During fractionation of the extracts from this strain, 1-minute fractions were collected. The UV elution profile was monitored and each fraction was assessed for toxicity (Fig. 2). With T-39, all the toxicity was eluted between 15 and 17 minutes.

Of the six Floridian strains (Babinchak et al., 1986), three strains were sufficiently toxic to permit testing. Figures 3, 4, and 5 illustrate the UV pro-

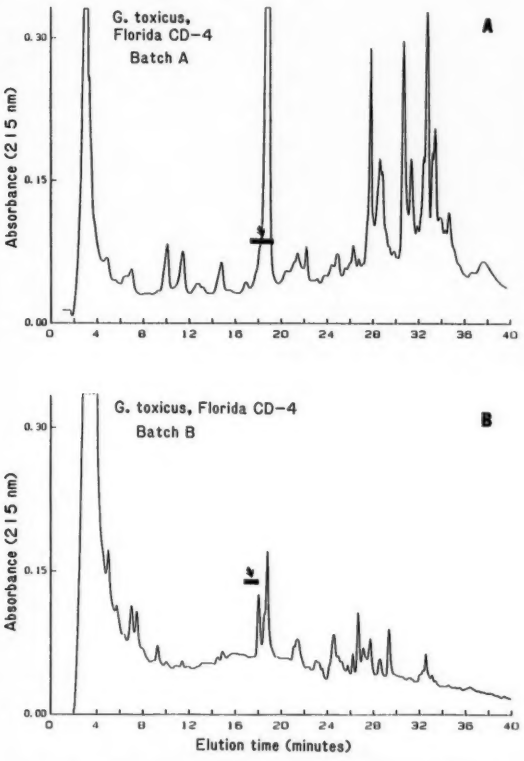

Figure 3.—Chromatographic profiles from two batches of cells of *G. toxicus*, clone CD-4, isolated from the Florida Keys. The two batches were grown under similar conditions, but at different times. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

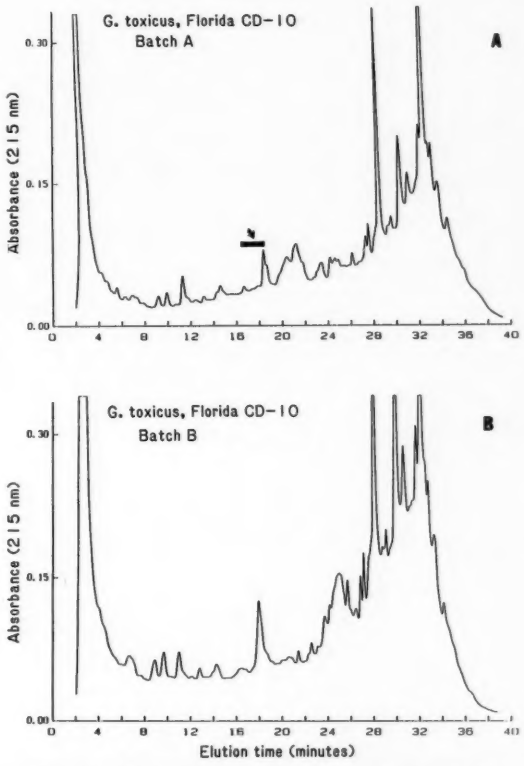

Figure 4.—Chromatographic profiles from two batches of cells of *G. toxicus*, clone CD-10, isolated from the Florida Keys. The two batches were grown under similar conditions, but at different times. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

files obtained from strains CD-4, CD-10, and CD-20, respectively. Panels A and B of Figures 3 and 4 represent the same respective strain but were extracts from two different cell "batches" harvested from cultures having similar culture conditions. The two UV profiles for CD-4 (Fig. 3A, B) showed very little similarity, while the two profiles for strain CD-10 (Fig. 4A, B) were almost identical. In both cases, however, toxicity was limited to the same fraction regardless of the UV profile pattern exhibited by the cellular constituents harvested from different culture batches. In all three Floridian strains, toxicity was limited to a single area eluting as fraction 17 and/or 18.

Figure 6 illustrates the UV profile obtained with the methanol extract of *Ostreopsis lenticularis*. All the toxicity was eluted with the solvent front, i.e., the eluant fraction that had little or no interaction with the column's stationary phase.

The relationship between the toxic component(s) of *G. toxicus* and the ciguatoxin in fish flesh is not well understood. Extracts of ciguatoxic fish from Caribbean waters were supplied by Joseph McMillan. The extracts were dried and prepared for HPLC in the same manner as the dinoflagellate extracts. The UV profile of a typical fish extract is presented in Figure 7. When individual fractions were tested for toxicity, only fractions 26 and 27 were positive in the mouse bioassay.

To determine if the toxicity in the Caribbean fish extract was ciguatoxin, an aliquot of purified ciguatoxin (Scheuer et al., 1967; Nukina et al., 1984)

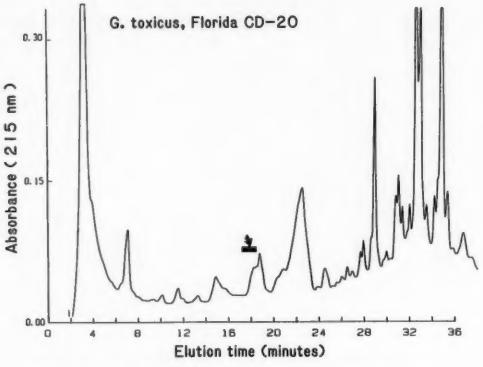

Figure 5.—Chromatographic profile of an extract of *G. toxicus*, clone CD-20, isolated from the Florida Keys. Bar indicates the eluant fractions with toxicity when 1-minute fractions were bioassayed.

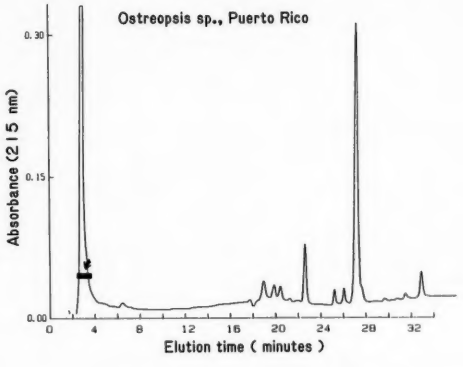

Figure 6.—Chromatographic profile of an extract of *O. lenticularis* isolated from Puerto Rico. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

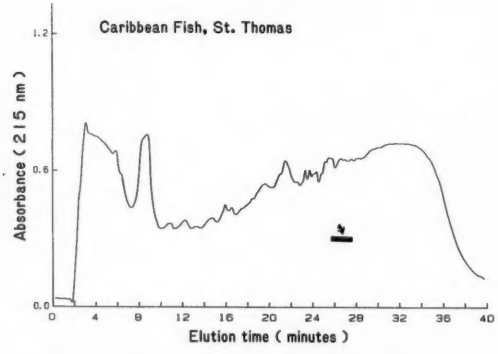

Figure 7.—Chromatographic profile of an extract of ciguatoxic fish caught around St. Thomas, U.S. Virgin Islands. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

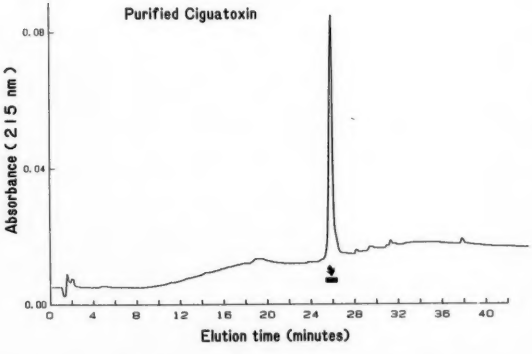

Figure 8.—Chromatographic profile of purified ciguatoxin. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

was dried, dissolved in aqueous methanol, and applied to the chromatographic system (Fig. 8). A single UV-absorbing peak could be detected in the eluant. Moreover, the mouse bioassay revealed a single fraction of toxicity which eluted in tube 26. Chromatography of a fish harvested from the waters near Tahiti revealed a distinctive UV profile (Fig. 9) and two areas of toxicity. One of the toxic fractions corresponded to the toxic fraction obtained with purified ciguatoxin (Fraction 26). A second toxic fraction was observed, and it was eluted in Fraction 29.

Table 2 summarizes the results of this study and defines the migration of the toxic components in terms of their time of elution relative to that of the phenol marker run immediately before and after the test sample. At least four distinct toxins were evident. A very polar toxin was detected in extracts of *O. lenticularis*. Laboratory cultures of *G. toxicus* produced a second toxin that was more polar than the third toxin detected, ciguatoxin. An additional nonpolar toxin was present in the Pacific fish sample; the Caribbean fish sample lacked this component.

Table 2.—HPLC elution time of toxicity relative to phenol (R_t).

Source of material	R_t[1] (min.)
O. lenticularis, Puerto Rico	0.44
G. toxicus T-39, Hawaii (Batch A)	2.25
G. toxicus T-39, Hawaii (Batch B)	2.14
G. toxicus CD-4, Florida (Batch A)	2.81
G. toxicus CD-4, Florida (Batch B)	2.62
G. toxicus CD-10, Florida (Batch A)	2.63
G. toxicus CD-20, Florida	2.74
Fish, St. Thomas	4.01
Fish, Tahiti	3.95 and 4.40
Purified ciguatoxin	3.94

[1]Ratio of the mid-point of the fraction(s) exhibiting toxicity to the retention time of phenol.

Discussion

Unfortunately, biologists have not had a reasonable means to distinguish the toxins associated with ciguatera. As a result, many broad-based assumptions have appeared. For example, it has been assumed that the toxin isolated from Caribbean fish involved in clinical cases of ciguatera is the same toxin originally defined by Scheuer et al. (1967) as ciguatoxin, even though toxin from a Caribbean fish source has never been purified and chemically characterized. This report presents data providing the first strong evidence that the toxin isolated from Caribbean fish may be the same chemical entity previously described as ciguatoxin. Obviously, definitive arguments will require the structural elucidation of the purified toxins from each of the two geographical sources.

In addition to ciguatoxin, another toxin was present in the Pacific fish sample. Interestingly, this toxin produced similar symptomology as purified ciguatoxin and the dinoflagellate toxin when administered i.p. to mice, indicating biological similarities among all three ciguatera-associated toxins. This very nonpolar toxin may be related to the second chemical form of ciguatoxin recently reported by Nukina et al. (1984). This less polar toxin may be similar to scaritoxin isolated from some toxic fish of the Pacific Islands (Bagnis et al., 1974) and which has been shown to interconvert to ciguatoxin in vitro (Nukina et al., 1984).

To date, unequivocal evidence has not been presented that the dinoflagellate, *G. toxicus*, when grown in the laboratory, contains ciguatoxin. Currently, an effort is being made to collect a sufficient quantity of cells of this dinoflagellate from their natural habitat to determine if "wild" cells of this organism produce detectable levels of ciguatoxin, as reported by Yasumoto et al. (1979). It is interesting to note that the Hawaiian strain may be producing a slightly more polar toxin than the Floridian strain; however, additional samples need to be analyzed before a statistical evaluation of any differences can be reported.

The method described herein should be viewed as a reliable means to provide preliminary and tentative identification of the ciguatera-associated toxins. This method is relatively simple to perform and does not require extensive purification of the toxin sample. The detection of biological activity purposely rests with the mouse bioassay, a very reliable and noncontroversial assay of toxicity when performed correctly. The inclusion of markers into the chromatographic runs insures uniformity of conditions and permits different laboratories an element of standardization. This is exceedingly important for those laboratories lacking the chemical expertise and/or the quantity of toxin necessary for purification. Hopefully, use of this or a similar method will result in a universally acceptable standard for defining those toxins potentially involved in ciguatera seafood poisoning.

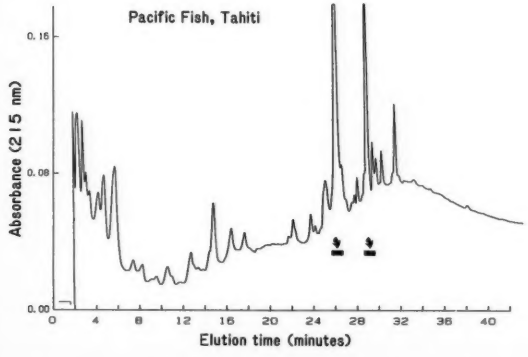

Figure 9.—Chromatographic profile of an extract of ciguatoxic fish caught around Tahiti. Bar indicates the eluant fraction with toxicity when 1-minute fractions were bioassayed.

Acknowledgments

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An HPLC-Fluorescence Method for Identifying a Toxic Fraction Extracted from the Marine Dinoflagellate *Gambierdiscus toxicus*

L. V. SICK, D. C. HANSEN, J. A. BABINCHAK, and T. B. HIGERD

ABSTRACT—A high performance liquid chromatograph using fluorescence detection was employed for quantitatively monitoring the toxic fraction extracted from the marine dinoflagellate, Gambierdiscus toxicus. *The toxin in this extract is probably a precursor of or closely related chemically to ciguatoxin, a toxin found in several species of reef fish and the cause of one of the more potent and treacherous toxicities that can result from the consumption of seafood. Using continuous flow, post-column derivatization of the chromatographically separated algal toxin, the method yielded statistically significant increases in analytical precision and decreases in time for analysis and detection limit compared with the more traditional mouse bioassay. Determination of molecular weight of the toxin extracted from* G. toxicus *indicated that this toxic material had a molecular weight of about 38,000 daltons.*

Introduction

Although there have been previous attempts to develop quantitative chemical assays for ciguatoxin, no analytical method, having high analytical precision and a low detection limit, presently exists. A radioimmunoassay method (Hokama et al., 1977) was reported using immunoglobulin isolated from sheep that had been immunized with a conjugate of ciguatoxin human serum albumin. The assay (Hokama et al., 1977) proved useful as a relatively crude screening technique for fish suspected of being ciguatoxic. Using the same antisera, an enzyme immunoassay was also recently reported to yield relatively accurate but nonquantitative detection of ciguatoxin in fish (Hokama et al., 1984, 1985). Emerson et al. (1983) used a simplified counterimmunoelectrophoretic assay using nonimmunized sera and were able to distinguish toxic from nontoxic fish flesh. The method, however, could not be used to quantitate toxin concentration.

Routine detection of ciguatoxin and possibly related toxins (e.g., maitotoxin) has been by bioassay methods. As summarized by Withers (1982), a variety of animals and tissues have been used to bioassay for ciguatoxin and toxin(s) extracted from the dinoflagellate *Gambierdiscus toxicus*. Such methods have included frog sciatic nerve tissue and whole animal assays using cats (Hessel et al., 1960), the mongoose (Banner and Boroughs, 1958), ducks (Ross, 1947), chickens (Larson and Rivas, 1965), turtles and crayfish (Banner et al., 1960), brine shrimp (Grande et al., 1976), and, most extensively used, the mouse (e.g., Ohshika, 1971). Although these bioassays have been nonquantitative assays, Sawyer et al. (1984) attempted to quantitate the mouse bioassay by constructing LD_{50} dose response curves.

The objective of the present study was to develop an HPLC method using fluorescence detection for detecting and quantitating the toxic fraction extracted from the dinoflagellate *G. toxicus*.

Materials and Methods

Based on previous results indicating that ciguatoxin and its presumed progenitor *G. toxicus* toxin absorb light within UV or IR ranges relatively poorly (Tachibana, 1980), experimentation and instrumentation were designed to investigate fluorescence absorption as a means of analytically detecting these toxins. Previously, Tachibana (1980) reported relatively weak UV absorbance of semi-purified ciguatoxin at 215 nm. This absorbance was partially attributable to some chromophore associated with the toxic moiety. Because ciguatoxin, either purified or in the form of a crude solvent extract from fish tissue, was not available for the present study, methanol extracts from *G. toxicus* were used.

An extract was prepared by concentrating cultures of *G. toxicus* via filtration and freeze-drying the cells. A known weight of dried cells was extracted with 80 percent aqueous methanol for 48 hours using a wrist arm shaker. Extracts were weighed and brought to a volume of 1 ml with 80 percent aqueous methanol. Culture conditions for growing *G. toxicus* were as described by Babinchak et al. (1986). A stock solution of *G. toxicus* extract used to construct a calibration curve had a concentration, in mouse units (MU), of 1.21 MU/μl. A MU was defined as the dry weight of methanol extracted *G. toxicus* necessary to produce a statistically computed LD_{50} when intraperitoneally injected into ≈ 20 g mice. The calibration curve for toxic extract was linear in a range of 15-200 MU, had a slope of 0.428, a Y intercept of 13.08, and a regression coefficient of 0.987. Purified saxitoxin, used to standardize analytical response, was obtained from the U.S. Food and Drug Administration. Toxic extract was chromatographically

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separated from the crude methanol extract of *G. toxicus* using a DuPont[1] model 820 high performance liquid chromatograph (HPLC). An Alltech 25 cm column packed with 10μ, CN (cyano) substrate was used.

An initial effort was made to separate chromatographically precolumn, derivatized *G. toxicus* extract. Many biological toxins, such as paralytic shellfish toxins, form fluorescent derivatives when oxidized (Buckley et al., 1978). Oxidized derivatives of *G. toxicus* that had been semipurified by HPLC were formed by alkaline oxidation. Periodic acid and ammonium hydroxide were used to achieve batch mixtures of alkaline oxidized derivatives using 0.065 M and 2.0 M concentrations, respectively. The reagents and sample were mixed for about 1 minute and maintained at pH 9.8. Maintenance of pH was critical to insure oxidation of substrate without obtaining a large quantity of precipitation. Before injecting the sample for HPLC analyses, the pH of the oxidized sample was adjusted in the range of 5.0-5.5 with 6.0 M acetic acid to maximize fluorescence detection (Sullivan and Iwaoka, 1983). Elution of a toxic fraction was achieved using a 20-80 percent water-methanol linear gradient run at a ramp rate of 4 percent/minute. An Aminco-Bowman Spectrophotofluorometer was used as a fluorescence detector. All analyses were conducted using excitation and emission wavelengths of 340 and 410 nm, respectively.

Post-column derivatization was accomplished using an adaptation of a continuous-flow system designed by Sullivan and Iwaoka (1983) for analyses of paralytic shellfish toxins. Three reagent reservoirs containing periodic acid, ammonium hydroxide, and acetic acid (0.065 M, 2.0 M, and 4.0 M, respectively) were connected to the post-column effluent line (Fig. 1). Ammonium hydroxide and acetic acid were pumped using a Technicon Auto Analyzer peristaltic pump, while periodic acid was pumped using a Milton Roy LDC high pressure pump. The reaction coil consisted of 0.56 mm i.d. stainless steel tubing with an internal volume of ≈ 1.5 ml. Residence time, using a column flow rate of 1.8 ml/minute, was ≈ 60 seconds. Flow rate for each reagent was regulated by a series of check valves and clamps to insure a pH of 9.0-9.8 in the reaction coil and 5.5-5.7 before the mobile phase entered the fluorometer. Optimum reagent flow rate, temperature, and pH of both the reaction coil and fluorometer cell for greatest analytical precision are given in Figure 2. The optical chamber of the spectrophotofluorometer was fitted with an American Instrument Company 9 μl continuous flow cell, Model J4-7484. Mobile phase A was HPLC grade 80 aqueous percent methanol and mobile phase B was a potassium phosphate buffer (pH 7.2). A linear elution gradient of 20-80 percent aqueous methanol was programmed at a ramp rate of 4 percent/minute, a 1-minute delay after initial injection and a 5-minute hold at the end of the gradient.

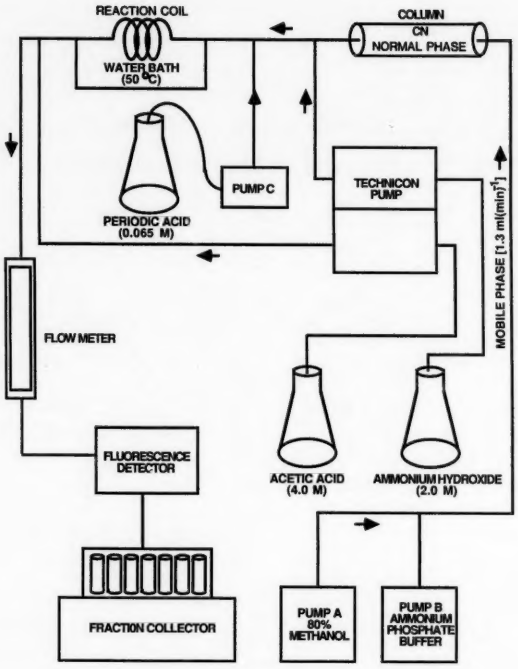

Figure 1.—Schematic diagram of the HPLC-fluorescence system including the post-column derivatization apparatus.

The molecular weight of the toxic moiety extracted from *G. toxicus* was estimated by first separating the toxic fraction chromatographically. The toxic fraction was eluted from a CN column, after the post-column apparatus had been disconnected, and 2-minute fractions collected with a Gilson fraction collector. The elution gradient was the same as described for post-column derivatization. The toxic fraction, identified from post-column derivatized fluorometry by corresponding retention time, was dried under nitrogen and brought to a volume of 20 μl with 80 percent aqueous methanol. Using a Varian gel permeation column (TSK Gel SW 2000 in tandem with a TSK Gel SW 3000, each 30×0.75 cm, Fair and Sick (1984)) and the same elution gradient as

[1]Reference to trade names or commercial firms does not imply endorsement of the National Marine Fisheries Service, NOAA.

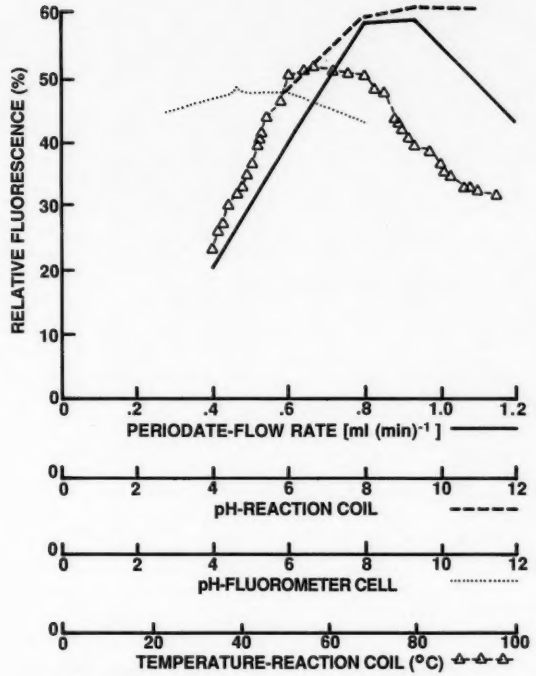

Figure 2.—Selected parameters that were examined to obtain optimum fluorescence detection of *Gambierdiscus toxicus* toxin extracted (from a stock solution having a concentration of 1-12 MU/μl). Subsample size injected for all tests was 5 μl.

Table 1.—Results obtained using HPLC-fluorescence versus mouse bioassay to quantitatively analyze a stock solution of *Gambierdiscus toxicus* extract.

Toxin conc. in dilutions[1] from stock (MU/20 μl)	Toxin conc. by HPLC[2] (MU/20 μl)	Toxin conc. by mouse[3] (bioassay (MU/20 μl)
1.00	1.15±1.3	0
2.75	3.1±0.8	4.55±7.1
3.4	3.9±0.7	5.5±0.1
4.2	5.3±0.6	10.73±5.6
5.0	4.8±0.3	7.50±2.5
5.5	6.1±0.8	17.24±12.7
13.8	14.9±2.7	45.4±21.6
Mean	5.61	14.42
Estimate of analytical precision[4]	6%	0.5%
Estimate of detection limit[5]	0.81 MU	9.7 MU

[1]The concentration of toxin (as estimated from measurements of toxicity) in a stock solution of *G. toxicus* extract was determined using five replicate mouse bioassays (four mice per assay) of a given volume of the stock extract. Concentrations given were established using dilutions from the stock extract solution having a concentration of 1.12 MU/μl. Stock solution was made from *G. toxicus* extract having a concentration of 20.1 MU/mg dried extract. *G. toxicus* extracts were obtained from stock cultures averaging 12,600 cells/mg dry weight.
[2]Values are the average and standard deviation from five duplicate injections made up from the stock *G. toxicus* extract.
[3]Values are the average and standard deviation based on three replicate intraperitoneal injections of a given volume of *G. toxicus* stock solution. Each respective volume injected was adjusted to correspond to established concentrations cited in column one.
[4]Analytical precision was defined as the reciprocal of the variance among seven replicate determinations and expressed as a percentage.
[5]Detection limit was defined as the sample concentration yielding a peak area having twice the standard deviation of a series of blanks.

previously described, the toxic fraction was separated on the basis of molecular weight exclusion. The elution profile for respective fractions collected was calibrated for molecular weight using albumin (66,000), ovalbumin (43,000), ribonuclease A (13,700), phenylalanine (165), and histidine (151) as standards.

Data expressed as percent of total fluoroscence was converted to mouse units of toxicity by constructing a calibration curve comparing percent fluorescence versus toxicity (from mouse bioassays). Analytical standardization of the curve, subsequent conversion to toxicity units, and statistical treatment of the data are described in Table 1. LD_{50} values and subsequent MU values and constants for mouse bioassays were determined using a "time-to-death" dose response analysis based on four mice for each dose. Using the results of this bioassay, toxicity was calculated as follows:

$$MU = [K_1]\,[(TD)^{K_2}]^{-1},$$

where

K_1 = 80.07,
K_2 = 1.41, and
TD = time-to-death.

Analytical precision for the method for the dose response curve, using subsamples from a stock solution, was 2.35 percent.

Results

Attempts to tag extracted toxins via spiking the primary HPLC mobile phase (potassium phosphate buffer) with aniline, a commercially available fluorophore, resulted in fluorometrically detectable peaks and acceptable baseline noise. Using a methanol extract of *G. toxicus* known to be toxic, based on mouse bioassay, retention time of the fluorometrically detectable peak coincided with the area of the chromatogram previously demonstrated to have the toxic moiety. However, reproducible, quantifiable results using the standard additions technique were not obtained. In addition, the analytical precision (defined as the reciprocal of the variance among seven replicate determinations

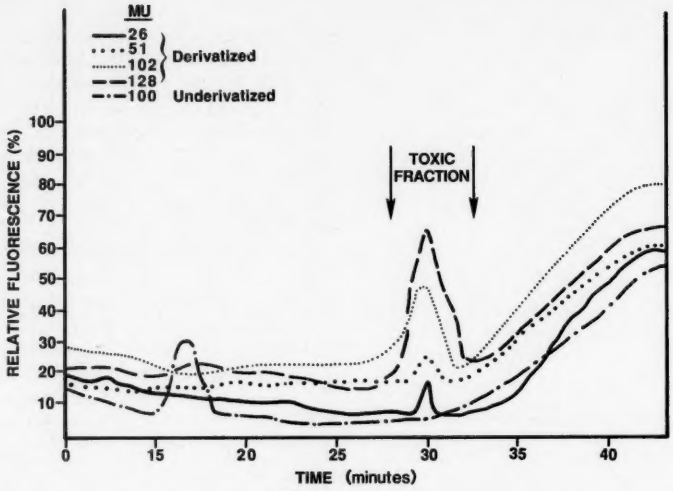

Figure 3.—Chromatographic comparison of HPLC-fluorescence separations of underivatized versus precolumn derivatized extracts from *Gambierdiscus toxicus*. Extracts used for these comparisons had an average dry weight of 0.068 ± 0.009 mg/MU.

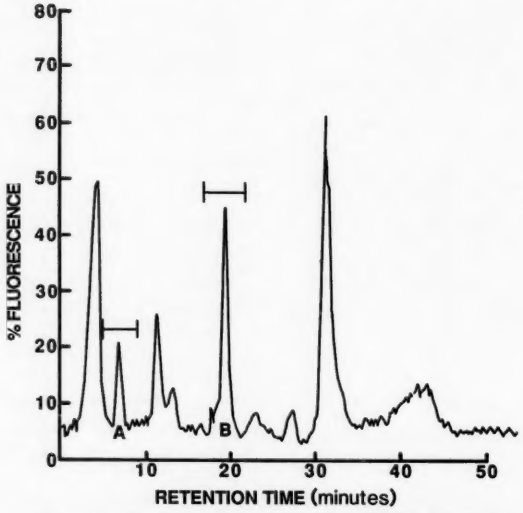

Figure 4.—Chromatographic results from HPLC-fluorescence analyses of *Gambierdiscus toxicus* extract using continuous-flow, post-column derivatization. Peaks identified by mouse bioassay as being toxic were A, saxitoxin standard, and B, peak assumed to contain the toxic moiety of *G. toxicus* extract. The chromatograph was obtained using a 5 µl subsample taken from a stock extract solution having a concentration, estimated from mouse bioassay, to be 1.12 MU/µl.

and expressed as a percentage, Steel and Torrie (1960)) of the technique was much poorer than obtained with the mouse bioassay. The calculated detection limit (defined as the sample concentration yielding a peak area having twice the standard deviation of a series of blanks) was >200 MU.

Derivatives of *G. toxicus* extract, formed in batch preparations by alkaline oxidation, were subjected to HPLC fractionation and the eluant detected fluorometrically (Fig. 3). Although fluorescence response was linear for extract doses >26 MU, attempts to detect concentrations of toxic extract <26 MU resulted in nonreproducible peaks and were calculated to be below the calculated analytical detection limits of 26.82 MU.

Using HPLC separation and post-column derivatization of a combined standard of saxitoxin and *G. toxicus* extract, relatively sensitive fluoremetric detection of corresponding toxic fractions from both saxitoxin standards and crude methanol extracts of *G. toxicus* was obtained (Fig. 4). Based on mouse bioassays of 2-minute fractions, the toxic moiety from *G. toxicus* extracts eluted in a fluorometrically detectable peak at ≈ 18 minutes. Similarly, a purified saxitoxin fraction, identified by mouse bioassay, eluted in a well resolved peak at 6-7 minutes. Based on a calibration curve established with the time-to-death mouse bioassay, the estimated detection limit by HPLC analysis for crude *G. toxicus* toxin was ≈ 0.81 MU (Table 1). Detection limit for the mouse bioassay, using the time-to-death analysis, was calculated to be 9.70 MU.

Molecular weight analyses, using HPLC size exclusion chromatography, indicated that the toxic fraction of *G. toxicus* extract had a molecular weight of 38,000 to 40,000 daltons. This analysis was conducted using *G. toxicus* extract that had been fractionated by HPLC-affinity chromatography and then refractionated by size exclusion chromatography.

Discussion

Toxin extracted from *G. toxicus* was considered appropriate for use in the

present study even though the ultimate research objective concerns development of a rapid, highly sensitive analytical method for detecting ciguatoxin. First, *G. toxicus* extract may in fact be the toxin described by Yasumoto et al. (1976) as maitotoxin and may be closely related to ciguatotoxin. Second, Sawyer et al. (1984) have demonstrated that a *G. toxicus* extract injected intraperitoneally into mice yields physiological symptoms that are empirically indistinguishable from those observed in mice injected with ciguatoxin. In addition, a physiological response thought to be specific for ciguatoxin, a lowering of body temperature by ≈ 10°C (Sawyer et al. 1984), was also observed in mice injected with *G. toxicus* extract.

Several attempts have been made to develop fluorescence detection methods for marine biotoxins. For example, Buckley et al. (1978) were able to separate oxidized derivatives of paralytic shellfish toxins by HPLC and detect toxic eluents using fluorescence detection. Sullivan and Iwaoka (1983), Sullivan et al. (1983), and Sullivan and Wekell (1984) have used alkaline oxidized derivatives of paralytic shellfish toxins to fluorometrically detect toxic fractions eluted from a HPLC column at concentrations of <1 MU.

Although parameters used for post-column derivatization of PSP toxins (Sullivan and Iwaoka, 1983) were generally adaptable for post-column analyses of *G. toxicus* extracted toxin, several differences in parameterization were found (Fig. 2). Using the dimensions recommended by Sullivan and Iwaoka (1983) for the reaction coil and other components of post-column tubing (Fig. 1), approximately twice the flowrate (0.81 vs. 0.42 ml/minute) of the oxidant, periodic acid, was required for derivatization of *G. toxicus* extract relative to derivatization of PSP toxins. Optimum derivatization of *G. toxicus* extract occurred using a reaction coil temperature of 55°-60°C. In contrast, Sullivan and Wekell (1984) recommended a reaction coil temperature of 75°C. Perhaps the use of more oxidant for *G. toxicus* extract derivative formation, relative to that used for derivatization of PSP toxins, resulted in overoxidation of algal toxin at temperatures appropriate for PSP derivatives. Similarly, using a column flow rate of 1.3 ml/minute, as reported by Sullivan and Wekell (1984) for PSP post-column derivatization, resulted in poorly resolved peaks. Maximum peak resolution and detection sensitivity for *G. toxicus* extracted toxin was obtained in the present study using a column flow rate of 1.5-2.0 ml/minute.

A comparison between HPLC and mouse bioassay methods for detecting and quantitating *G. toxicus* extract indicated that the HPLC method yielded greater precision, lower detection limits, and significantly lower average mean values than the mouse bioassay method (Table 1). The detection limit for *G. toxicus* extracted toxin, as determined for HPLC analyses, was 0.81 and 9.70 MU for the mouse time-to-death analysis. At a concentration of 1 MU (20 μl/injection), for example, detection of *G. toxicus* extract was obtained by HPLC, but no detection of toxin was obtained by mouse bioassay (Table 1). Analytical precision was 6 percent for HPLC analyses and 0.5 percent for the bioassay technique. A two-tailed T test between means of the results from the HPLC and mouse bioassays indicated that the mouse assay yielded significantly higher results at the 95 percent confidence level. Similarly, results of the two assay methods were found to be significantly different at the 95 percent confidence level using analysis of variance.

Although the chemical nature of purified *G. toxicus* toxin is not known, the toxic moiety analyzed in this study is probably the same toxin or closely related to the toxin reported as maitotoxin by Yasumoto et al. (1976). Maitotoxin was more polar than ciguatoxin when isolated from the surgeonfish *Ctenochaetus striatus* (Yasumoto et al., 1976). Based on chemical and physiological characteristics, Yasumoto et al. (1976) speculated that maitotoxin was either identical or closely related to an ichthyotoxin produced by the dinoflagellate, *Prymnesium parvum*. In addition, Yasumoto et al. (1984) suggested that the molecular weight of maitotoxin is significantly larger than that of ciguatoxin (1000 to 1500 daltons (Tachibana, 1980)). In the present study, *G. toxicus* toxin was determined to have a relatively large molecular weight (≈ 38,000 daltons), was relatively polar (eluted rapidly in a water-methanol gradient), and was the only toxic fraction found in the solvent extract of *G. toxicus*.

Use of an HPLC-fluorescence technique for monitoring toxicity offers a monitoring method having the sensitivity necessary for conducting further ciguatoxin research as well as monitoring the potential health risks of consuming selected seafood. Because ciguatoxin and maitotoxin are recognized as among the most potent, nonproteinaceous toxins (Tachibana, 1980), concentrations of toxin used in most experimental designs and concentrations lethal to test organisms and to humans are in the nanogram range. Higerd (1984), for example, estimated that if man is assumed to be twentyfold more sensitive to ciguatoxin than the mouse, a dose of as little as 1.8 μg would be lethal. Even using only semi-purified preparations of toxin, as in the present study, it was necessary to monitor as little as 50 μg (the determined LD_{50} of *G. toxicus* extract for mice) to conduct experiments using *G. toxicus* extract. If *G. toxicus* extract is used in purified form for developing assays, such as proposed immunological studies that could evolve into "marketplace" screening procedures for ciguatoxin fish (Higerd, 1984), the ability to monitor the toxic extract at nanogram concentrations by HPLC-fluorescence, as demonstrated in the present study, could be an essential tool.

Oxidation products formed in the alkaline oxidative derivatization of *G. toxicus* extract have not been determined. The fluorescent compound formed by peroxide oxidation of a PSP toxin, saxitoxin, was determined by Wong et al. (1976) to be an aminopurinyl propionic acid derivative. Furthermore, gonyautoxin, another PSP toxin, was found to yield more than one fluorescent product when oxidized (Shimizu et al., 1976). Both derivatized products formed from gonyautoxin were similar to aminopurinyl propionic acid. Although the chemical structure of ciguatoxin and maitotoxin are not known, their olefinic-polyether nature

(Tachibana, 1980) may render them structurally similar to the PSP toxins.

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Response of Mice to *Gambierdiscus toxicus* Toxin

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Introduction

Ciguatera seafood poisoning is a serious human illness brought on by ingesting certain coral reef-associated fish in tropical and subtropical regions. The toxin carried by these fish was first isolated by Scheuer et al. (1967). The presence of the toxin has been confirmed in red snapper, *Lutjanus bohor*, moray eel, *Gymnothorax javanicus*, amberjack, *Seriola aureovittata*, and others. Toxic fish probably acquire ciguatoxin through their diet, and that one source of toxin is probably the benthic dinoflagellate, *Gambierdiscus toxicus*, found in association with certain macroalgae of coral reefs (Yasumoto et al., 1977). Extracts of laboratory grown cultures of *G. toxicus*, however, have yielded a more polar toxin similar to maitotoxin described from surgeonfish, *Acanthurus* sp. (Yasumoto et al., 1979). The relationship of this dinoflagellate toxin to ciguatoxin is unknown, though one possible explanation is that the dinoflagellate toxin becomes chemically converted as it is metabolized during transfer through the marine food web.

ABSTRACT—Response to toxins extracted from cultured Gambierdiscus toxicus *was evaluated in mice. Toxin preparations administered intraperitoneally or intravenously gave similar dose-response curves, whereas oral administration elicited no response. Time-to-death determination was dose dependent and was quantitated to the dose-response based on lethality. The 48-hour lethality dose curves for the dinoflagellate toxin were comparable to those previously published for ciguatoxin extracted from fish, whereas the time-to-death curves showed a strong difference. The LD_{50} response of scheduled multiple injections suggested a 4- to 8-hour half-life for toxin activity in the mouse model. Sex and strain of the mouse did not affect susceptibility.*

Toxin(s) extracted from cultured *G. toxicus* injected intraperitoneally (i.p.) into laboratory mice is reported to evoke gross symptoms indistinguishable from those reported for partially purified fish extracts containing ciguatoxin (Hoffman et al., 1983; Sawyer et al., 1984). Both toxins exhibit similar dose-response curves and both elicit in mice a striking hypothermia, which is reversed by increasing ambient temperature (Sawyer et al., 1984). It is important, therefore, that the biological and chemical relationship between these toxins be clarified. In this investigation, we have utilized the mouse bioassay to examine further the biological activities of the dinoflagellate toxin for comparison with ciguatoxin and to gain an estimation of the biological half-life of *G. toxicus* toxin in mice.

Materials and Methods

Cells of *G. toxicus*, Adachi and Fukuya, were supplied by Rick York of the Hawaii Institute of Marine Biology. The isolate, clone T-39, was cultured in 100-liter vats in F/2 medium supplemented with a seaweed extract. Harvested dinoflagellate cells were shipped to South Carolina in aqueous methanol, and upon arrival, cells were extracted for 7 days at room temperature in methanol:water (80:20). The suspension was clarified by centrifugation, the supernatant dried, and the resulting solids weighed and dissolved in absolute methanol. This suspension was filtered, designated as crude dinoflagellate extract, and stored at 4°C.

Assays for toxicity were conducted on ICR female, ICR male, and C57BL/6 female mice all weighing approximately 20 g each. Animals were maintained on Wayne Laboratory Animal diets (Lab-Blox)[1] and water, ad libitum. A known quantity of the crude dinoflagellate extract was resuspended in phosphate-buffered saline (PBS) containing 5 percent Tween-80 and administered (in 0.2 ml aliquots unless otherwise stated) to mice intraperitoneally, intravenously (i.v.), and by gavage. Control animals received an equal volume of the solvent vehicle. Lethality was recorded at 48 hours for some studies, and time-to-death after injection was recorded for other studies. One mouse unit (MU) of toxicity is defined as that quantity of crude extract capable of eliciting a fatal dose in half of the test animals by 48 hours. Body temperatures were recorded using a YSI rectal probe (Model 43TA, Yellow Springs Instrument Co., Yellow Springs, OH) within 2 hours after administration to obtain early indications of toxicity.

For the retention time study, a stock solution of dinoflagellate extract (13.3 MU/ml) was prepared in PBS containing 5 percent Tween-80 (67 μg extract/ml). Each ICR female mouse was administered 0.15 ml (10 μg) i.p. of the stock solution or 0.15 ml of a 1:3 dilution; a reduced volume was used due to administration of multiple injections. One control group received 10 μg of tox-

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[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

ic extract as a single injection, and another control group received 3.3 μg in a single injection. Each test animal in the eight remaining groups received three equally spaced injections over a time interval ranging from 0 to 30 hours between individual injections. Lethality was recorded after 48 hours of the last injection.

Results and Discussion

Sex and Strain Differences

Determination of the dose-response relationship to the crude dinoflagellate extract administered i.p. to ICR female, ICR male, and C57BL/6 female mice suggested that sex and strain of the mouse did not markedly influence toxicity (Fig. 1). The LD_{50}'s for ICR female, ICR male, and C57BL/6 female mice were estimated to be 260 μg/kg, 393 μg/kg, and 192 μg/kg, respectively. ICR females were used in later tests.

The i.p. injection of ciguatoxin extracts into laboratory mice has been a reliable bioassay method for ciguatera-associated toxins and has been widely adopted by investigators of this field. The variability that may be present in the assay due to the sex and strain of mice used has not been studied. The results of this limited study indicated that no apparent difference should be expected in dose-response with respect to sex and strain of the test animal.

Route of Administration

To determine the effect of the route of administration on the toxicity of *G. toxicus* extract, animals received the toxin in three ways: Intraperitoneally, intravenously, and orally (p.o.). Response of mice to 10 MU of toxin administered by i.p. and i.v. routes was uniformly fatal (Table 1). Average time-to-death, however, was much shorter following i.v. injections. Administration by gavage of approximately 20 MU resulted in no test mice fatalities.

The potency of the dinoflagellate toxin, as defined by percent fatalities of the treated mouse populations at 48 hours, was not significantly different between mice receiving the dinoflagellate extract i.p. or i.v. However, mice administered the toxin i.v. responded earlier (time-to-death) than mice injected i.p. The toxicokinetics of the dinoflagellate and fish toxins are not known. It is interesting that the time to develop overt symptoms, such as hypothermia in mice (Sawyer et al., 1984) or clinical symptoms in man (Bagnis et al., 1979), occurs over a period of hours. In view of the high lipophilicity of these toxins, which should enhance their equilibration across membranes and promote their accessibility to the site(s) of action, a much more rapid response, perhaps within minutes, would be expected.

The lack of any response, including temperature depression in mice administered the dinoflagellate extract by gavage, raises interesting questions regarding the toxin's rate of absorption, physical or biochemical inactivation, etc. Preliminary studies (not reported), in which the toxicity of the dinoflagellate extract was not lost when incubated under acidic conditions or with various mouse tissue extracts, suggest that simple physical or enzymatic inactivation was not responsible for abrogation of dinoflagellate toxicity when administered orally. The observation that the route of administration influenced the dose-dependence of the toxicity was first made by Baden[2] and is in contrast to the animal response with fish toxin (putative ciguatoxin) described by Banner et al. (1960), in which an equivalent response was obtained whether mice were injected with toxic fish extracts or fed the extract equivalent to twice the injected dose.

Determination of Mean Death Time

A standardized mouse bioassay has been universally accepted to determine the toxicity of saxitoxin, a marine toxin of dinoflagellate origin and the causitive agent of paralytic shellfish poisoning. The method described by Schantz et al. (1957) involved determination of median death time for a given dilution of a suspect extract injected i.p. relative to a saxitoxin standard. For ciguatoxin, Tachibana (1980) reported the relationship between the dose of toxin and time-to-death in the mouse. For comparison with these published reports, we observed the lethality of the dinoflagellate toxin as judged by time-to-death response in relation to the 48-hour LD_{50} response (Fig. 2). Groups of test mice were injected i.p. with dinoflagellate extract equivalent to 2-33 MU. At these doses, death occurred between 2 and 16 hours, and the mean time-to-death was

[2]Baden, D. Department of Biochemistry, University of Miami School of Medicine, Miami, FL 33101. Personal commun., 1982.

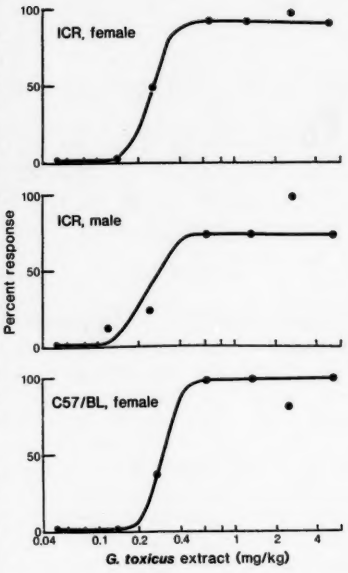

Figure 1.—Percent response (death) in 48 hours displayed by three mouse strains to i.p. administration of *G. toxicus* extract diluted in PBS. Data points represent a minimum of 8 animals.

Table 1.—The effect of administration route on lethality of *G. toxicus* extract.

Administration route	Amount	No. of animals[1]	Percent response[2]	Time to death (h)
I.p.	50 μg	6	100	4 to 22
I.v.	50 μg	6	100	1 to 3
Gavage	100 μg	4	0	

[1]Female ICR mice.
[2]Percentage of fatalities after 48 hours.

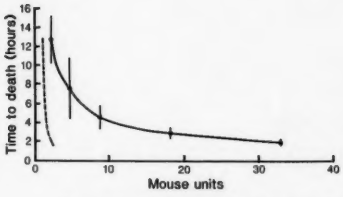

Figure 2.—Relationship between *G. toxicus* extract dose and time-to-death of ICR female mice (solid line). Mice received doses expressed in equivalents of mouse units (1 MU = 5.2 μg extract). Error bars indicate one standard error of the mean (n = 8). Curve defined by the dotted line represents the administration of fish ciguatoxin as reported by Tachibana (1980).

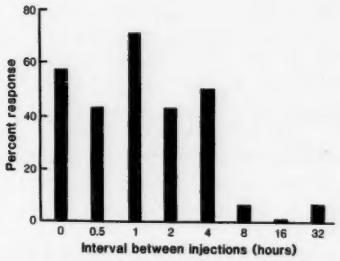

Figure 3.—Percent response (death) within 48 hours following three equally spaced i.p. injections of *G. toxicus* extract.

dose dependent. A nonlinear equation estimated the relationship between the independent variable (dose) and the dependent variable (time-to-death). Using the Simplex method for determining the values of the parameters by least squares, the following equation gave a reasonable approximation of the curve in Figure 2:

$$\text{Dose (MU)} = 0.8\ (\text{time-to-death in hours})^{-1}.$$

Coupled with differences in sensitivity to orally administered toxins, the marked difference in time-to-death response to ciguatoxin reported by Tachibana (1980) and that obtained in our study with *G. toxicus* extracts further illustrates that the two toxins may not be equivalent biologically. The dashed line in Figure 2, superimposed on the dinoflagellate response curve, represents a curve based on the formula reported by Tachibana (1980) for ciguatoxin:

$$\text{Log dose (MU)} = 2 \log (1 + \text{time-to-death in hours})^{-1}.$$

The differences between the fish-toxin-derived curve and the dinoflagellate-derived-curve are evident from this figure.

Estimation of Toxin Retention Time

Our knowledge regarding the ability of the animal to clear, sequester, or otherwise inactivate ciguatera-associated toxins is vitally important to our understanding of the human illness, ciguatera. Unfortunately, direct methods of determining the biological fate of these toxins is not readily available. To gain insight as to the "half-life" of the dinoflagellate toxin, portions of an otherwise lethal dose were administered over several time intervals in order to extrapolate the time point in which the effect of individual doses were no longer additive (Fig. 3). A control group which received a single 10 μg standard dose of the toxin extract elicited the expected 75 percent lethality response. Another control group received a single 3.3 μg dose and exhibited no fatalities. Groups that received three injections of 3.3 μg each at time intervals ranging from 0 to 4 hours between injection gave between 40 and 60 percent response, while those animals in groups injected at intervals between 8 and 30 hours gave less than 10 percent response. From these results, it appeared that an estimation of biological half-life for this toxin in the mouse model lies somewhere between 4 and 8 hours, and that this toxin may not accumulate in the body.

The persistence for months of the neurological symptoms associated with ciguatera poisoning in humans certainly suggests that either ciguatoxin is retained and remains active for long periods of time or that the damage caused by ciguatoxin is not quickly repaired. As yet, a similar "half-life" study using crude extracts of ciguatera fish has not been performed. It will be of interest to know if ciguatoxin, unlike the dinoflagellate toxin, is retained in the mouse model.

Biological studies of the toxins associated with ciguatera have been minimal. However, many of the biological properties described can be very useful in defining the various toxins that have been isolated. As a clear understanding of the biological activities of these ciguatera-associated toxins is obtained, their relationship to each other and a more rational approach to minimizing the impact of ciguatera may become evident.

Acknowledgments

We thank Marilyn Orvin for her technical assistance, Rick York for supplying extracts of the dinoflagellate, and H. Hugh Fudenberg for valuable discussions. This research was supported in part by NOAA Grants NA80AA-D-00101 and NA84A-H-SK098.

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Thecal Analysis of the Tropical Benthic Dinoflagellate *Gambierdiscus toxicus*

ALFRED R. LOEBLICH III and STEPHEN R. INDELICATO

ABSTRACT—The theca of the benthic dinoflagellate, Gambierdiscus toxicus, *has been determined, by the means of the chloral hydrate-hydriodic acid-iodine staining technique, to consist of the following plates: pp, 4', 6", 6c, 8s, 5"', 2"". The thecal plate overlap pattern, fission line, and position of the ventral pore were used to determine thecal plate homologies rather than relying strictly upon the relative position of a plate before assigning plates to a particular series.* Gambierdiscus toxicus *is considered to be closely related to members of the genera* Coolia *and* Ostreopsis *and the species of these three genera are placed in the family Ostreopsidaceae. These generic assignments are based on the possession of common morphological and biochemical characteristics.*

Introduction

The dinoflagellate, *Gambierdiscus toxicus*, a causative agent in ciguatera poisoning, was originally described from material collected from the Gambier Islands in the Pacific Ocean (Adachi and Fukuyo, 1979). The earliest detection of this species in the Atlantic Ocean was by Sousa e Silva (1956) from plankton collected from the island of Boavista, Cape Verde Islands, in October and November 1948. She referred to this species as an unidentified *Goniodoma*. Along the coastal United States, *G. toxicus* has only been detected along the Florida Keys (Taylor, 1979; Bergmann and Alam, 1981; Besada et al., 1982). No detailed analyses of the morphology of the American isolates of *G. toxicus* have been made, and the only illustration of material collected in continental American waters is a single scanning electron microscopic view of an hypotheca (Bergmann and Alam, 1981).

The analysis of *G. toxicus* thecae by Adachi and Fukuyo (1979) of Gambier Island material and Taylor (1979) of Hawaiian material did not encompass a study of plates from disassociated thecae. This technique is necessary to reveal details of the sulcal series as well as spatial relationships of plates in the various series. We present an analysis of the thecae of a Florida isolate using the chloral hydrate-hydriodic acid-iodine staining technique (Stosch, 1969). Our assignment of plates to particular series is based on plate homologies determined by: Overlap patterns, path of the fission line, presence and position of a ventral pore, in addition to the common method of plate enumeration, size, and position. We have not relied strictly on plate position in the theca as the criteria for assigning them to a series, rather choosing to determine most probable homologies of plates when comparing two species. We have previously used these characteristics to reveal homologies in gonyaulacoid and peridinioid plate patterns (Loeblich, 1984; Loeblich and Loeblich, 1979), and have applied this methodology here in an analysis of *G. toxicus*.

Materials and Methods

Gambierdiscus toxicus used in this study was isolated from algal detritus in the intertidal region of the Straits of Florida, Windley Key, Fla., in August 1983. Cells of a clone (F-8) were grown in unialgal culture at 27°C in a 12:12 light dark photoregieme under a light intensity of 300 ft.c. Culture medium used was a modified GPM medium (Loeblich, 1975), with salinity increased to 33‰, soil extract deleted, and nitrate and phosphate concentrations reduced by one-third. Chloral hydrate-hydiodic acid-iodine theca stain was used as described by Stosch (1969). Photomicrographs were taken on a Leitz Orthoplan[1] microscope equipped with an Orthomat camera.

Results and Discussion

Our findings suggest a thecal tabulation for *G. toxicus* of: Pore plate (pp), apical (4'), precingular (6"), cingular (6c), sulcal (8s), postcingular (5"'), and antapical (2""). Illustrated in Figure 1 is the plate nomenclature for *G. toxicus*, the thecal plate overlap pattern where detected, and the fission line for this species. The path of the fission line for *G. toxicus* was determined earlier in our laboratory by Besada et al. (1982). It separates the theca into an anterosinistral moiety with the plates pp, apical, 1", 2", 1"', 2"', 3"', 1c, 2c, 3c, and a posterodextral moiety with the plates 4c, 5c, 6c, 3", 4", 5", 4"', 5"', antapicals. The path of the fission line among the sulcal plates remains to be determined.

In the epitheca, plates of the posterodextral moiety overlap those of the anterosinistral moiety, while in the hypotheca, plates of the anterosinistral moiety overlie those of the posterodextral moiety. The overlap pattern was

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[1]Reference to trade names or companies does not imply endorsement by the National Marine Fisheries Service, NOAA

determined from the differential marginal growth in the sutures of two adjacent plates (Fig. 2). The overlap pattern could be deduced from observations of the differential intercalary growth at the margin of the dorsalmost plates of the precingular and postcingular series. These two dorsal plates overlap the adjacent plates in their respective series as well as the apical and antapical plates that border them. The wide growth band on the plate edges of thecae from older cells was used to determine that plates with these wide bands were overlying adjacent plates when viewed from the cell's exterior.

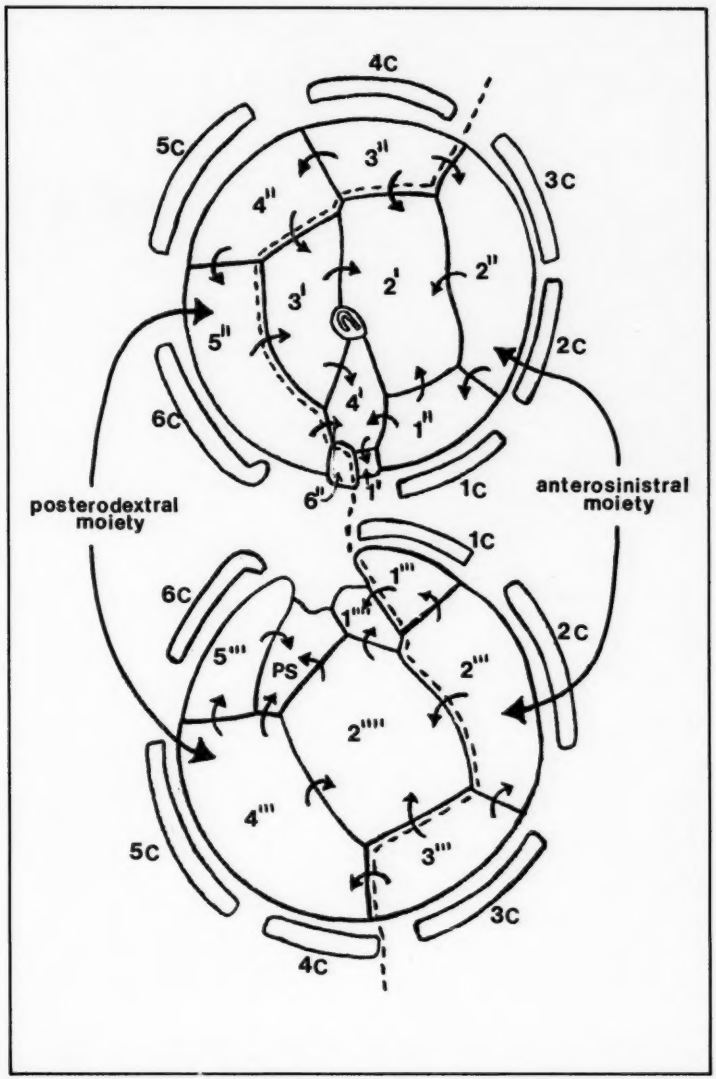
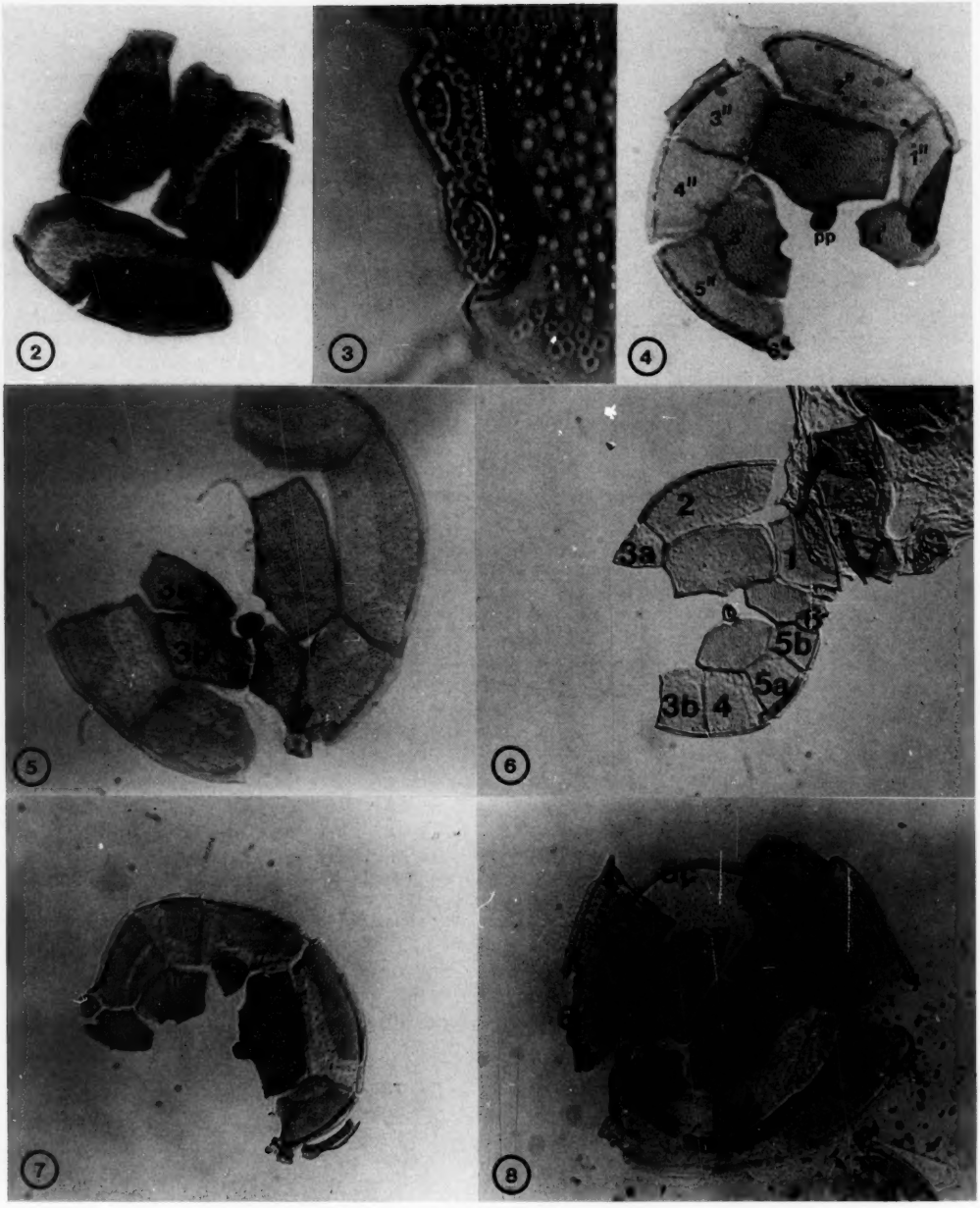


Figure 1.—Diagram of the theca of *Gambierdiscus toxicus* depicting the fission line (dashed), and the overlap pattern (small arrows indicate the direction of plate overlap), epitheca above, hypotheca below.

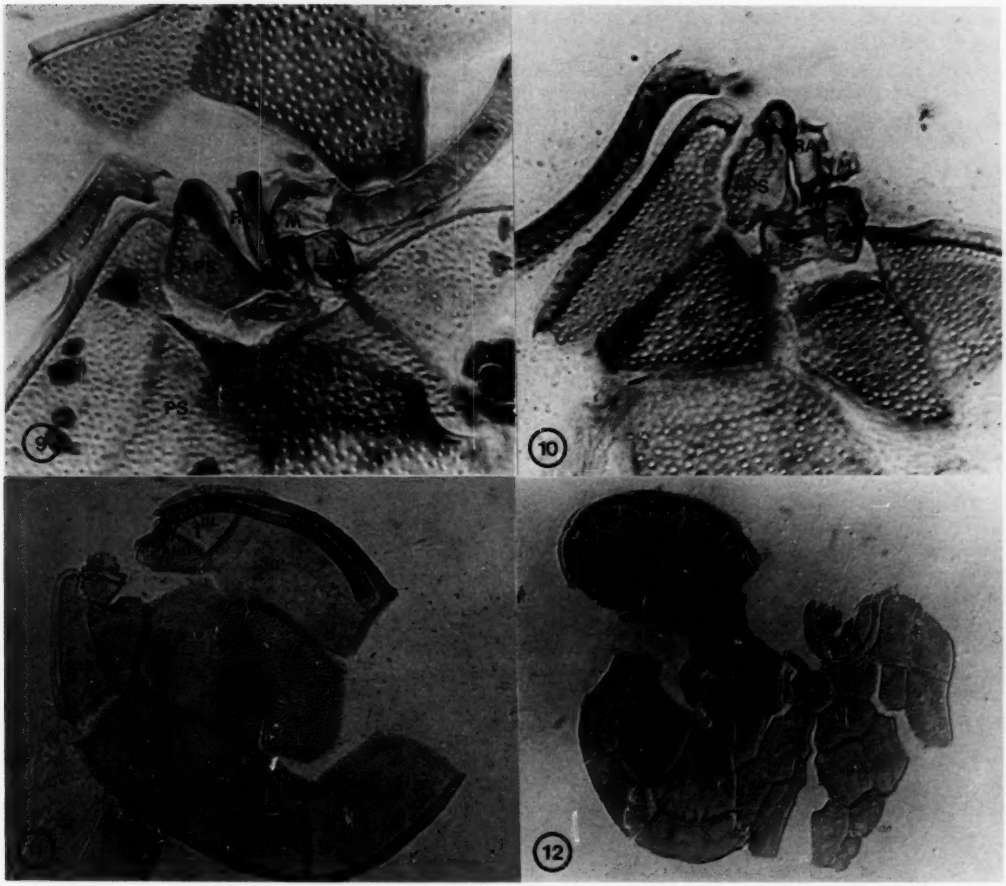
The apical pore has a curved slit in the plate that produces a tongue-like projection that is directed toward the third apical plate. An aberrant specimen was found with two apical pores (Fig. 3). An apical pore in the form of a slit is a feature this genus has in common with gonyaulacoids. A ventral pore is also present between plates 6'' and 1'. The above described thecal pattern with its associated plate assignments is gonyaulacoid in nature. See Loeblich and Loeblich (1979) for a discussion of gonyaulacoid nomenclature.

The epitheca (Fig. 4) has three large plates surrounding a pore plate; these are interpreted as three apical plates. The ventral side of the epitheca has two small plates in a precingular position. The smaller of the two plates we interpreted as an apical plate located in a position displaced from the apical pore. The other small plate we interpreted as the last member of the precingular series. The path of the fission line can be used to support the interpretation that there is an apical plate in a precingular position. Fission lines in other species (e.g., *Ostreopsis ovata*, Besada et al., 1982) separate the epithecae such that only the first two precingular plates are partitioned to the anterosinistral moiety. Thus in *G. toxicus*, the extra plate on the anterosinistral moiety is more properly interpreted as a member of the apical series. Figure 5 illustrates an aberrant specimen that has five apical plates due to an extra suture splitting the third apical plate.

The ventral pore in *G. toxicus* lies between the two plates in a precingular position that we interpret as the first apical and the last precingular. The ventral pore when present in gonyaulacoids and dinophysioids is most commonly associated with two apical plates, the first and last. Thus the position of the ventral pore in *G. toxicus* suggests that at least one of the two plates that borders the pore is a homolog of an apical plate despite its position in a precingular location. Illustrated in Figure 6 is a specimen with eight precingular plates; ex-



Figures 2-8.—Chloral hydrate-hydriodic acid-iodine stained theca of *Gambierdiscus toxicus*: Figure 2, marginal growth bands; Figure 3, aberrant theca with two apical pores; Figure 4, the epithecal plates; Figure 5, an aberrant epitheca with an extra suture splitting the third apical plate into two smaller plates (3a and 3b); Figure 6, an aberrant epitheca with eight precingular plates. Plates partitioned by the extra sutures are the third and fifth precingular plates; Figure 7, an aberrant epitheca with an anterior intercalary plate (1a); and Figure 8, an epitheca with the six cingular plates (c).



Figures 9-12.—Chloral hydrate-hydriodic acid-iodine stained theca of *Gambierdiscus toxicus*: Figures 9 and 10, the sulcal series with anterior (as), right anterior (ras), right posterior (rps), left anterior (las), left posterior (lps), posterior (ps) and two medial (m) plates; Figure 11, a hypotheca with a portion of the sulcal plates attached; and Figure 12, an aberrant theca considered to result from incomplete cytokinesis, note the two apical areas (p).

tra sutures occur between the third and fourth precingular plates and between the fifth and sixth precingular plates. The additional sutures can be deduced by comparing the plates that these precingular plates contact in adjacent plate series. Figure 7 illustrates an aberrant cell with an anterior intercalary plate.

The cingulum of *G. toxicus* is composed of six plates (Fig. 8), with the fifth cingular being the largest plate in this series. The last plate of the series (6c) curves downward at its distal end into the sulcus. The sutures of the cingular series are collinear with those of the precingular and postcingular, except for the suture between 2c and 3c, which occurs in the middle of plate 2" and the junction of 4c and 5c, which occurs in the middle of plate 4"'.

The pre- and postcingular plate series are each composed of five large plates separated by four sutures, while the six relatively equal cingular plates are separated by five sutures generating a non-collinear suture of the cingulum on the epitheca and hypotheca (Fig. 1).

The sulcus is composed of six large plates and at least two smaller internal plates (Figs. 9, 10). We were unable to find the two small plates (labelled Sar and Sal) that Adachi and Fukuyo (1979) found at the anterior of the sulcus. Perhaps these could be interpreted as thickening along the margin of the plate where the anterior sulcal contacts the

plates we designated as the 6" and 1'. Taylor (1979) designated the last member of the postcingular series as a sulcal plate and the left anterior sulcal plate as the first postcingular plate, generating a postcingular series of seven plates. Taylor (1979) also designated the posterior sulcal plate as a third antapical plate. The overlap pattern suggests that this plate in question is the posterior sulcal as it underlies the 5"' and 2"" plates as would be expected of a posterior sulcal plate. Additionally, using Taylor's interpretation of this plate, no other plate in this genus could be interpreted as a posterior sulcal plate. We feel this designation does not reflect obvious thecal homologies when comparing *G. toxicus* to other dinoflagellates. We prefer to consider all plates lining the sulcal region as sulcal plates, especially those that occur within the sulcal depression.

We find the hypotheca (Fig. 11) to have five postcingular plates rather than the interpretation of six plates in this series as suggested by Adachi and Fukuyo (1979) and Taylor (1979). These investigators designate a very small plate located in the sulcal depression on the left side of the sulcus as the first postcingular plate. Such an interpretation is inconsistent when one compares the thecal plate assignments of peridinioids and gonyaulacoids. In peridinioids the plate in this position in the sulcus is a sulcal plate. In an effort to reveal the homologies between the theca of a variety of dinoflagellate genera, we consider those plates that are in the sulcal depression and form a complex encircling the flagellar pore region as sulcal plates. Thus, in our interpretation of the hypotheca of *G. toxicus* we find eight sulcal plates, five postcingular, and two antapical plates.

The hypotheca is interpreted to have two antapicals, the larger of the two located at the posterior of the cell (Fig. 11). We interpret this species to have two antapical plates rather than an antapical and a posterior intercalary plate. This interpretation better reveals homologies of the hypotheca, particularly when comparing peridinioids and gonyaulacoids. Support for interpreting the gonyaulacoid plate commonly referred to as the posterior intercalary plate as an antapical is derived from the pattern of plate overlap on the hypotheca of both peridinioids and gonyaulacoids. In both these lineages the plate we have labelled as the second antapical overlaps the plate designated as the first antapical. The pattern of overlap of these two plates suggests to us that these two plates in both lineages are homologous plates. The difference in the hypotheca between the peridinioids and gonyaulacoids is in reality only one of the realtively greater size of the gonyaulacoid second antapical in relation to the first.

Our analyses support the hypotheca plate arrangement of Adachi and Fukuyo (1979) in finding that the plate we refer to as the second antapical does not contact the last (5"') postcingular plate. In contrast, Taylor (1979) illustrated a plate he considers the 3"" that does not completely separate his 6"' and 2"" plates. As both Pacific (Adachi and Fukuyo, 1979) and Atlantic (described here) forms of *G. toxicus* have an identical hypothecal arrangement, Taylor's (1979) illustration of the hypotheca plates on the cell's right is most likely erroneous.

Depicted in Figure 12 is the theca of a cell that is difficult to interpret. It could represent a cell similar to the line drawing that Taylor (1979) presented as illustrating a zygote. An alternate conclusion that we prefer is that this specimen represents an aberrant cell that did not complete cytokinesis but formed a composite wall consisting of thecal plates that would have surrounded the two daughter cells. The presence of two apical pores on this specimen suggests that two thecae are involved, resulting from cell fusion or incomplete cytokinesis.

Assigning plates to particular series is only an aid in identifying and comparing species. We feel that the assignments should be made to reveal the greatest number of similarities between dinoflagellates from different genera and lineages.

What is important is to be consistent from species to species in choosing a number and series to denote the plate. As an aid in making these decisions we recommend that as many criteria as possible should be used in addition to the widespread reliance on the position of the plate in the theca, e.g., overlap pattern, fission line, and thecal pores (see Loeblich (1984) for a discussion of the homologies of the dinoflagellate theca). An emphasis on detecting the homologies in the theca of one species when compared to another would have avoided the divergent thecal formulas proposed by Adachi and Fukuyo (1979), Taylor (1979), and Besada et al. (1982) for *G. toxicus*.

Gambierdiscus toxicus has a plate pattern that is very similar to the *Ostreopsis* and *Coolia* spp. These species differ in: 1) The relative size of the plates; 2) the shifting of the sutures in these genera, which has resulted in variations in the relative sizes of the plates; and 3) the position of the plates such that a particular plate may contact different plates in the different genera. Despite these thecal differences, a basic, common thecal pattern is apparent in these benthic dinoflagellates.

Interestingly, the sterol composition (Besada, 1982; Loeblich and Loeblich, 1983) and internal anatomy (Besada et al., 1982) also reveal that these species are closely related. The synthesis of dinosterol and cholesterol and their presence in a 1:2 ratio, respectively, in both *Ostreopsis ovata* and *Coolia monotis* suggests that these two genera are closely related and supports Lindemann (1928) in placing *Coolia* in the synonymy of *Ostreopsis*. *Gambierdiscus toxicus*, while possessing a similar ratio of these two sterols, differs in having the additional sterol, 24-methylcholesterol, suggesting that it is more distantly related to *Coolia* and *Ostreopsis* than they are related to each other.

All members of these three genera possess spirally coiled fibers that are vesicle bound (Besada et al., 1982). The absence of these structures in other dinoflagellates and the thecal plate similarities strongly suggest that these three genera are very closely related and perhaps should be considered congeneric. We consider them members of the family Ostreopsidaceae and place them in the gonyaulacoid lineage of families.

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Identification of the Photosynthetic Pigments of the Tropical Benthic Dinoflagellate *Gambierdiscus toxicus*

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Introduction

Gambierdiscus toxicus, a marine benthic dinoflagellate, is currently of interest to toxicologists since it has been found to produce toxins that have been implicated in ciguatera poisoning (Yasumoto et al., 1977, 1979). While numerous reports have focused on the structure and mechanism of action of the toxins associated with *G. toxicus*, relatively few have addressed fundamental questions regarding the nontoxin biochemistry and physiology of this organism.

In this paper we add additional data that can be used by others in characterizing their *G. toxicus* strains as well as in comparative studies with other dinoflagellate species. Pigment composition of microalgae has been used as a taxonomic criterion for a number of years (Strain et al., 1944; Goodwin, 1952; Riley and Wilson, 1967; Norgard et al., 1974). Studies on chloroplast pigment patterns of photosynthetic dinoflagellates have assisted biologists in grouping these organisms on biochemical data (Jeffrey et al., 1975) in addition to the classical groupings based on morphology.

There has been one published study on the chloroplast ultrastructure coupled with data for some of the pigments of *G. toxicus* (Durand and Berkaloff, 1985). In their study, however, the carotenoids were not completely characterized, either qualitatively or quantitatively. Additionally, they report the unusual occurrence of chlorophyll c_1. In this paper we identify the major photosynthetic pigments of the Florida isolate of *G. toxicus* and compare them with the data for the Pacific strain of this species.

ABSTRACT—Photosynthetic pigments of the Florida isolate of Gambierdiscus toxicus *were investigated to aid in characterizing this strain and to assist in comparisons with Pacific Ocean isolates. The pigments were separated using thin-layer chromatography (TLC). Tentative pigment identifications were made from visible absorption maxima (in two solvents) and partition coefficients (hexane: 95 percent methanol). The TLC revealed the presence of 10 pigment bands. The chlorophylls* a *and* c_2 *were the major chlorophylls present. The major carotenoid was peridinin, followed in abundance on a weight basis by diadinoxanthin, dinoxanthin, and* B*-carotene.* Gambierdiscus toxicus *also contained a water soluble peridinin-chlorophyll* a*-protein complex. A trichromatic method was used to quantify the amount of total carotenoids, chlorophyll* a*, and chlorophyll* c*. The Florida isolate of this species differs from the published data for the Pacific isolate of this species in having only the* c_2 *form of chlorophyll* c *and qualitatively more carotenoids.*

Materials and Methods

Gambierdiscus toxicus was isolated from an intertidal environment on the southern coast of Florida by A. R. Loeblich III in 1983 and designated strain F8. Strain F8 was later grown for pigment analysis in 1.5 liter batches of GPM medium (Loeblich, 1975) adjusted to 31‰ salinity in 2.8 liter Fernbach flasks[1]. The previous paper in this conference (Loeblich and Indelicato, 1986) explains culture procedures and conditions.

Cultures were harvested by continuous-flow centrifugation at the end of the exponential phase of growth. The resulting cell pellet was sonicated in acetone and periodically shaken to facilitate the extraction of the chloroplast pigments. The pigment-containing acetone extract was repeatedly drawn off the cell debris, and fresh acetone was added until the acetone fraction was nearly colorless. The acetone extract was then briefly centrifuged to remove particulate cell debris from the preparation and evaporated to dryness under a stream of nitrogen at less than 40°C.

Dried pigments were dissolved in 180 µl of carbon disulfide and spotted repeatedly onto activated silica gel thin-layer chromatographic (TLC) plates, using 20 µl micropipettes. Development took place in a mixture of hexane/acetone (6:4) in a sealed chamber.

Developed plates were scanned at 470 nm using a Helena Quick-scan R & D scanning densitometer. The readout was used to provide an accurate means of locating the center of each pigment band to aid in the calculation of R_f values. The densitometer integrated the areas under the peaks from which relative percentages for each carotenoid were calculated.

Pigment fractions were then dissolved in ethanol or acetone. An absorption spectrum for each pigment fraction was produced over the visible light range

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

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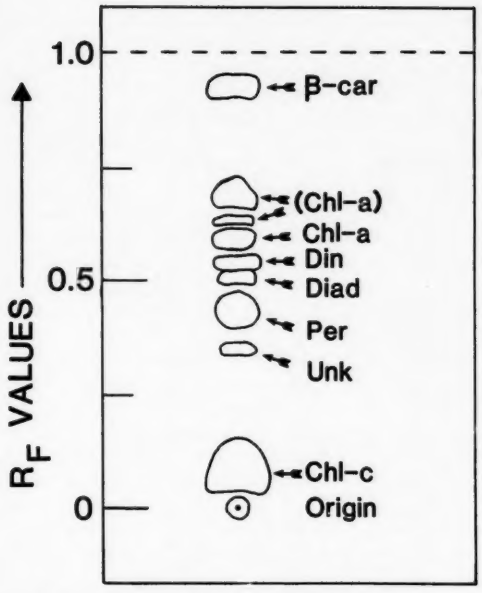

Figure 1.—Representation of a thin-layer chromatographic plate showing separation of *Gambierdiscus toxicus* photosynthetic pigments.

Table 1.—*Gambierdiscus toxicus* chloroplast pigment R_f values and absorbtion maxima.

Pigment[1]	Color	R_f value[2] (acetone: hexane)	Absorption maxima[3] (ethanol) (acetone)
Origin	Brown	0.00	454, 590, 665 No data
Chlorophyll-c	Grass-green	0.09	445, 587, 636 450, 583, 633
Unknown	Brown-green	0.35	No data
Peridinin	Red-orange	0.44	473 470
Diadinoxanthin	Yellow-orange	0.51	[4](409), 431, 457 (413), 438, 460
Dinoxanthin	Yellow	0.52	(405), 429, 457 (404), 429, 456
Chlorophyll-a	Green	0.56-0.57	413, 504, 535, 615, 666 412, 505, 535, 615, 666
(Chlorophyll-a)	Gray-green	0.62	410, 506, 535, 613, 669 No data
(Chlorophyll-a)	Gray-green	0.64-0.66	413, 510, 540, 612, 670 412, 507, 536, 610, 668
B-carotene	Yellow	0.91-0.98	429, 451, 478 (404), 429, 453, 475

[1]Pigments are listed in order of increasing mobility.
[2]These values were determined using a developing solvent consisting of 40 parts acetone and 60 parts hexane.
[3]For each pigment, the absorbtion maxima as measured in ethanol are on the first line and the absorbtion maxima as measured in acetone are on the second line.
[4]Absorption maxima given in parentheses are values for shoulders in the spectrum which could not be defined as a clear peak.

(350-750 nm) using a Beckman model 35 spectrophotometer. Partition coefficient values were determined, using the method of Petracek and Zechmeister (1956), to aid in the identification of pigments. The trichromatic method of Jeffrey et al. (1966) was employed to obtain values for the relative weight percentages of carotenoids and chlorophylls present in a whole-cell extract (95 percent acetone) prepared from the cells of *G. toxicus*.

Results

Thin layer chromatography revealed the presence of 10 pigment fractions extracted from the cells of *G. toxicus* (Fig. 1). Of these 10 fractions (numbered in order of elution), four had obvious chlorophyll affinities (fractions 9, 4, 3, 2), four were carotenoids (fractions 7, 6, 5, 1), and two others (fractions 8, 10) were unknown. The chromatographic and spectral properties of these fractions are presented in Table 1.

The major pigment within the cells of *G. toxicus* as measured by percent composition is chlorophyll-*a* (fraction 4) (Table 2). Spectral properties of two other fractions (2, 3), which are gray-green and have slightly higher R_f values than chlorophyll-*a*, suggest that these are degradation products of chlorophyll-*a*. Together, chlorophyll-*a* and its two degradation products make up 47.6 percent by weight of the total pigments in this species (Table 2).

Chlorophyll-*c*2 (fraction 9) is also found in large amounts in *G. toxicus* cells, constituting 16.33 percent of the total pigment weight (Table 2). This pigment's color is grass-green and was far less mobile than chlorophyll-*a* when chromatographically developed in an acetone:hexane solvent (Fig. 1). There was no evidence of chlorophyll-*c*1 in this strain of *G. toxicus*.

Of the four carotenoid pigments found in *G. toxicus*, peridinin (fraction 7) was present in the greatest amount. Peridinin constituted 23.0 percent of the total cellular pigments and 63.6 percent of the total carotenoids by weight (Table 2). Peridinin is easily recognized by its bright red-orange color and its characteristic broad absorption maximum at around 473 nm. It is the last carotenoid to be eluted during chromatographic separation in an acetone:hexane solvent (Fig. 1).

Table 2.—Percent total pigments, percent total carotenoids, and carotenoid partition coefficients for *Gambierdiscus toxicus*.

Pigment	Percent total pigments	Percent total carotenoids	Partition coefficient (hexane: acetone)
Chlorophyll-a	47.6		
Chlorophyll-c2	16.3		
Peridinin	23.0	63.6	3:97
Diadinoxanthin	4.9	13.6	5:95
Dinoxanthin	4.9	13.6	6:94
B-Carotene	3.3	9.1	

A yellow pigment fraction, which was the most mobile of all pigments contained in *G. toxicus*, and which traveled with the solvent front (R_f value = 0.91-0.98), was identified as B-carotene. Of the major pigments of *G. toxicus*, B-carotene was found to constitute only 9.1 percent of the total carotenoids and 3.3 percent of the total pigment content (Table 2).

The two major yellow xanthophylls produced by this organism had nearly identical spectral properties and R_f values (Table 1, Fig. 1). The first of the two to develop during chromatographic separation was yellow-orange and partitioned between hexane and 95 percent methanol in the ratio of 5:95 (hexane: methanol) (Table 2). This pigment has been identified as diadinoxanthin. The second xanthophyll to elute was bright yellow and had a partition coefficient ratio of 6:94 (hexane:methanol) and has been identified as dinoxanthin. Based on densitometric scan data, both xanthophylls are found in approximately equal amounts in the cell, together composing 9.8 percent of the total pigment content and 27.2 percent of the total carotenoids of *G. toxicus* by weight (Table 2). See Table 3 for the carotenoid and chlorophyll pigment ratios on a weight and molar basis.

Table 3.—Pigment ratios for *Gambierdiscus toxicus*.

Pigments	Wt. ratio	Mol. ratio
Chlorophyll-a:Chlorophyll-c_2	2.91	1.98
Total chlorophyll:Total carotenoid	1.77	2.82
Peridinin:Chlorophyll-a	0.48	0.68
Total carotenoid:Chlorophyll-a	0.76	

Occasionally, after centrifugation, freeze-thawing, or filtration of *G. toxicus* cells, an orange water-soluble pigment appeared in the supernatant. From spectral data, this orange pigment has been identified as a peridinin-chlorophyll-*a* protein complex.

Discussion

Comparison of our data concerning the chloroplast pigment composition of *G. toxicus* with that of other dinoflagellate species reveals that *G. toxicus* possesses a pigment content which is very similar to that of other dinoflagellates belonging to the gonyaulacoid lineage. Chlorophyll-*a*, chlorophyll-c_2, peridinin, dinoxanthin, diadinoxanthin, and B-carotene have been found in all photosynthetic dinoflagellates of this lineage. Those species (belonging to the peridinioid lineage) that harbor a photosynthetic endosymbiont (Jeffrey et al., 1975) are atypical as some of the pigments may belong to the symbiont derived from a different algal division: e.g., fucoxanthin in *Peridinium balticum* (Tomas and Cox, 1973). Jeffrey et al. (1975) noted that all of the peridinin containing photosynthetic dinoflagellate species studied contained an unknown "pink" pigment, which remained at the origin during thin layer chromatography. Thin-layer chromatography of *G. toxicus* pigments revealed this same fraction (1), although in *G. toxicus* this pigment was brown. Spectral data and immobility in a nonpolar solvent suggest this to be the peridinin-chlorophyll-*a* protein complex. These protein-pigment complexes act in a light harvesting capacity (Prezelin and Haxo, 1976) and appear to be an integral part of the dinoflagellate photosynthetic apparatus. These photosynthetic complexes have been observed in other dinoflagellates such as *Glenodinium* sp. (Prezelin, 1976), *Gonyaulax polyedra* (Prezelin and Haxo, 1976), *Amphidinium carterae* (Haxo et al., 1976; Siegelman et al., 1976), *Ceratium furca* (Meeson et al., 1982), and *Heterocapsa* spp. (Watson and Loeblich, 1983).

Durand and Berkaloff (1985) reported the presence of both chlorophyll-c_1 and chlorophyll-c_2 in *G. toxicus*. Our results disagree as we found only chlorophyll-c_2. Presence of chlorophyll-c_1 and c_2 in dinoflagellates whose major carotenoid is peridinin has been seen in only one species, *Prorocentrum cassubicum* (Jeffrey, 1976); all other photosynthetic dinoflagellates have only chlorophyll *a* and chlorophyll-c_2. The absence of a second form of chlorophyll *c* in our isolate, and the lack of other reports of this pigment in any dinoflagellate that is morphologically related to *G. toxicus*, suggests strongly that the report by Durand and Berkaloff (1985) should be reconfirmed.

Additionally, *P. cassubicum* belongs to a dinoflagellate lineage that shows affinities to the dinophysioids rather than to the gonyaulacoids to which *G. toxicus* belongs. Durand and Berkaloff (1985) found no evidence for the presence of an internal symbiont as an explanation for the occurrence of the second form of chlorophyll *c*. There remains the possibility that the fraction they identify as "chlorophyll-c_1" is a chlorophyll degradation product that could result from photooxidation. Such degradation products may occur if pigments are not analyzed under reduced light conditions and in a nonoxidizing (nitrogen) atmosphere. The discrepancies between the pigment pattern for the Florida and Pacific isolates of *G. toxicus* suggest that it may be necessary to analyze more isolates before a clear understanding of the apparent variability can be reconciled.

Durand and Berkaloff (1985) reported only two carotenoids, the xanthophylls diadinoxanthin and peridinin, from the Pacific *G. toxicus*; their study dealt mainly with the ultrastructure and chlorophyll pigmentation. No carotenes were reported for the Pacific isolate. The apparent differences in the carotenoid pigmentation between the Florida and Pacific isolates may disappear when a more detailed analysis of the Pacific form is published.

The properties of fractions 2 and 3 are similar to pheophytin-*a*, a magnesium-deficient chlorophyll molecule, which has been reported in *Peridinium cinctum* (Strain et al., 1944) and in Pacific Gyre phytoplankton samples (Jeffrey, 1975). It is not known whether pheophytin-*a* occurs naturally or if it is a laboratory artifact.

Jeffrey et al. (1975), in a survey of dinoflagellate pigments, showed a range for peridinin, the major dinophycean carotenoid, of 54-68 percent of the total carotenoid fraction. The value of 64 percent which we recorded for *G. toxicus* is within this range. Similar results exist for the ratio of peridinin to chlorophyll-*a* and for the ratio of total carotenoids to chlorophyll-*a* where ranges of

0.32-0.50 and 0.60-0.74 are found, respectively. *Gambierdiscus toxicus* exhibits values of 0.48 for peridinin:chlorophyll-*a* and 0.76 for total carotenoids:chlorophyll-*a* (Table 3).

Although the relative percentages of pigments may vary from species to species, the basic components of the dinoflagellate photosynthetic apparatus are present in all species for which data is available; such is the case with *G. toxicus*. See Jeffrey et al. (1975) for a review of dinoflagellate pigmentation.

Acknowledgments

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Gambierdiscus toxicus from the Caribbean: A Source of Toxins Involved in Ciguatera

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ABSTRACT—Gambierdiscus toxicus, *an epibenthic dinoflagellate, was collected with detritus from the surfaces of several genera of macroalgae* (Acanthophora, Caulerpa, Dictyota, Halimeda, *and* Laurencia) *in the Caribbean Sea on the south side of St. Thomas, U.S. Virgin Islands. The detritus was fractionated by sieving and then pro cessed in the laboratory to give samples which were 99 percent* G. toxicus. *Extraction yielded lipid (PPT-A) and water-soluble (PPT-B) toxic components that were heat-stable and precipitated in cold acetone. Intraperitoneal injection of PPT-A or PPT-B caused signs in mice (hypothermia, reduced locomotor activity, reduced reflexes, cyanosis, breathing difficulties, convulsions, and death) very similar to those produced by chromatographically purified extracts of fish remnants implicated in human ciguatera intoxications, particularly the marked lowering of body temperature. The ciguatoxic fish extract (CTX), however, was water insoluble and did not precipitate in cold acetone. In several chromatographic systems, PPT-A and PPT-B showed strong similarities but both differed markedly in comparison to fish CTX. The toxins of* G. toxicus *thus may undergo structural transformation when passed through the food web to ultimately become the CTX in fish that causes ciguatera poisoning in humans.*

Introduction

Dinoflagellates are responsible for the biosynthesis of many toxic compounds, some of which cause fish kills during red tides and, after transmission through the food chain, toxic shellfish poisoning and ciguatera in humans (Steidinger and Baden, 1984). The postulation by Randall (1958) of a benthic microorganism as the source of toxin that caused ciguatera was followed by studies on detrital feeders and herbivorous fishes (Yasumoto et al., 1971, 1976; Yasumoto and Kanno, 1976).

An examination of gut contents of surgeonfish, *Acanthurus* sp., from the Pacific led to work on benthic detritus and the discovery of a previously undescribed benthic dinoflagellate (Bagnis et al., 1977; Yasumoto et al., 1977b), later identified as a new genus and species, *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979; Taylor, 1979). Indirect evidence such as the similarities in the fish species involved and the symptomatology of fish poisoning victims suggests ciguatera is the same problem circumtropically (Banner, 1976; Withers, 1982), but this has yet to be shown unequivocally. A parallel situation appears to exist for the toxicology of the putatively causative organism, *G. toxicus* (Ragelis, 1984).

We present here our results on the toxicity in natural populations of *G. toxicus* collected from the Caribbean Sea. These findings are compared with our data on ciguatoxic extracts from Caribbean fish and with reports on the toxicology of *G. toxicus* and reef fish from the Pacific Ocean.

Materials and Methods

Field Collection and Laboratory Processing of *Gambierdiscus toxicus*

Gambierdiscus toxicus was collected from the surfaces of macroalgae (*Acanthophora*, *Caulerpa*, *Dictyota*, *Halimeda*, and *Laurencia* spp.) at depths of 0.5-1.0 m near Range Key in Brewer's Bay on the south side of St. Thomas, U.S. Virgin Islands. The site is a sandy-bottomed, well-protected baylet adjacent to the College of the Virgin Islands where sea action is gentle.

We took periodic survey samples of macroalgae, carefully engulfing plants with plastic jars or bags to avoid dislodging surface material, to monitor the *G. toxicus* population levels via stereoscopic (40X) examination. For large-scale collection we employed a hand-held plastic bilge pump to draw up detritus from the algal surfaces while avoiding bottom sediment. The seawater-detritus mixture was separated by filtering through 106 and 45 μm stainless steel sieves. The residue from the latter was taken to the lab, mixed with seawater at 30°C (since field temperatures ranged from 28 to 33.5°C) and allowed to settle in white plastic trays (34 × 45 × 12 cm) under 15,000 lux of light.

In 8-12 hours, *G. toxicus* sorted themselves from the other settled detritus and formed visible, mucilagenous assemblages along the tray sides and floating in the seawater. Assemblage formation seemed to be enhanced by 1-2 hours of darkness before being easily siphoned with a Pasteur pipet and plastic tubing. The harvests were concentrated on a 45 μm sieve and the cells washed off with and stored in absolute methanol.

After accumulating two or three harvests each from several field collections the cells were counted before extraction.

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Six aliquots were taken after thoroughly mixing the methanol cell pool, diluted with water until a 30 µl sample could be scanned microscopically (100×) without difficulty under a 25 mm cover slip and slide, and the cells totaled with a hand counter. Each diluted aliquot was sampled and counted twice, the highest and lowest aliquot counts discarded, an average determined, and the total cell number estimated by back calculating to the total methanol volume in the cell pool. In our experience this technique of lab processing yielded dinoflagellate samples that were more than 99 percent *G. toxicus*, as verified during microscopic quantification, and very few cells remained behind in the detritus.

Toxin Extraction

Our procedure for the extraction and preliminary purification of toxins from *G. toxicus* (Fig. 1) is similar to that of Bagnis et al. (1980). After twice boiling under reflux in methanol, filtration of cell debris, and concentration by rotary evaporation, the extract was fractioned into chloroform and water soluble phases. The chloroform-soluble fraction was concentrated, 80 percent methanol added, extracted with hexane, the methanol concentrated, and acetone added. The acetone was twice chilled overnight at −20°C and cold filtered (Whatman No. 50[1]) to yield Filtrate A (FLT-A) and Precipitate A (PPT-A). FLT-A was then repeatedly ultrachilled overnight at −95°C and cold filtered until either precipitate ceased forming or was of marginal toxicity. The water soluble fraction was extracted with butanol, the butanol concentrated, and acetone added. The acetone was cold treated as above to yield Filtrate B (FLT-B) and Precipitate B (PPT-B). After concentration and weighing, PPT-A and FLT-A were stored in chloroform, PPT-B in water, and FLT-B in acetone at −20°C. Extracts from the flesh of Caribbean fish that had caused human ciguatera intoxications were prepared using a procedure different from Figure 1 and published elsewhere (McMillan et al., 1980; Hoffman et al., 1983).

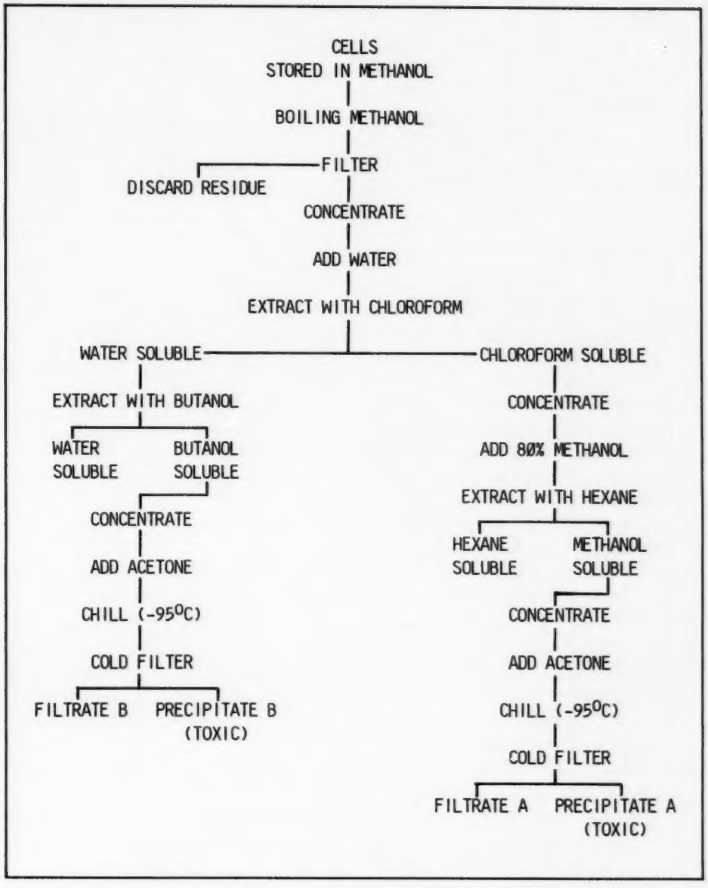

Figure 1.—Extraction and preliminary purification procedure for lipid-soluble (PPT-A) and water-soluble (PPT-B) toxins of *Gambierdiscus toxicus*.

Toxin Chromatography

The chloroform-soluble (PPT-A) and water-soluble (PPT-B) toxins from *G. toxicus* extraction and fish flesh ciguatoxin extract (CTX) were chromatographed in several systems. A silicic acid column (Mallinkrodt 100 mesh, activated at 100°C, 1.2 × 15 cm) was poured in chloroform and later a PPT-A or CTX sample in chloroform was added. PPT-B samples were dissolved in 0.5 ml of water with 0.5 g of silicic acid and freeze-dried. The silicic acid with the adsorbed PPT-B was then placed on the column. The column was washed with 90 ml chloroform and eluted with 90 ml each of chloroform: methanol at 95:5, 9:1, and 1:1 ratios, and methanol. The eluates were concentrated, dried under anhydrous N_2, weighed, and stored as above. For preparative thin-layer chromatography, glass plates precoated with 1.0 mm silica gel (Brinkman SIL G-100 UV 254 or Whatman PLK5F preadsorbent plates) were activated for 1-hour at 80°C and samples spotted in chloroform or water

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

(PPT-B). Four solvent systems were employed for development (see Table 2). Several R_f bands were scraped and eluted with chloroform:methanol at 3:1. *G. toxicus* sample plates were re-eluted with methanol. Bands from plates developed by partition TLC (System 4) were eluted with chloroform:methanol: water at 60:35:8.

Toxin Bioassay

A mouse bioassay, developed to screen fish flesh extracts for ciguatoxicity (Hoffman et al., 1983), was used to test cell and fish extracts and chromatographic fractions for toxicity. Test materials were dissolved in water or emulsified with 3 percent Tween 60 in water, injected i.p. into 16 to 24 g female mice (I.C.R., Medical University of South Carolina) and the minimum lethal dose determined in two to five mice.

Results

The yields and toxicity to mice of extracts from three samples of *G. toxicus* are given in Table 1. The variability of yield and toxicity per cell among the samples is probably due to unknown but natural causes since the same location, collection, and extraction procedures were used in all instances. We must emphasize, however, the importance of adequately ultrachilling and cold filtering the acetone filtrates to obtain the acetone precipitates (PPT-A and PPT-B, see Fig. 1). Sample 3 FLT-A, for example, was chilled (−95°C) overnight 11 times before it was only nominally toxic and PPT-A (toxic) ceased forming. Failure to chill cold enough and repeatedly may give the erroneous impression of appreciable toxicity in the filtrates. We interpret our results with this extraction procedure as yielding two toxic components, PPT-A and PPT-B. No toxicity was found in the hexane soluble or water soluble (after butanol extraction) fractions. A comparison of the biochemical properties of toxins from *G. toxicus* and reference ciguatoxic fish flesh is presented in Table 2.

PPT-A and PPT-B evoked signs in mice that were virtually indistinguishable from those caused by fish CTX (Hoffman et al., 1983), except there was infrequent salivation, only occasional lacrimation, and no diarrhea. Lower rectal temperature (normal 35°-38°C, toxin-treated 30°-31°C), reduced locomotor activity, reduced reflexes (pain, pinnal, corneal, withdrawal), cyanosis, breathing difficulties, convulsions, and death were manifest consistently. Convulsions, however, were observed much less frequently with PPT-B. Also, in contrast to fish CTX, cell toxins caused, within 1 hour after injection, a loss of body tone and intense vasodilation, which later progressed to cyanosis.

Table 1.—Yield and bioassay data of extracts from three samples of *Gambierdiscus toxicus* from the Caribbean Sea.

Extract and sample number[1]	Yield		Lethality in mice[2]	
	Total (mg)	ng per cell	mg per 20 g	Cells × 10^4 per 20 g
PPT-A 1	73.6	1.6	0.1	6.3
2	80.4	1.6	0.1	6.3
3	163.6	2.8	0.04	1.4
FLT-A 1	204.5	4.5	30.0 NL	666.6
2	259.2	5.0	25.0 NL	500.0
3	244.7	4.2	15.0	357.1
PPT-B 1	67.2	1.5	1.0	66.6
2	36.7	0.7	1.0	142.8
3	250.3	4.3	4.0	93.0
FLT-B 1	57.5	1.3	30.0 NL	2,307.7
2	54.3	1.0	30.0	3,000.0
3	93.7	1.6	30.0 NL	1,875.0

[1]See Figure 1 and Materials and Methods section for extract designations. Sample 1 = 44.9 × 10^6 cells, Sample 2 = 51.0 × 10^6 cells, and Sample 3 = 58.0 × 10^6 cells.
[2]NL = not lethal.

Table 2.—Comparison of solubilities and chromatographic properties of toxins from *Gambierdiscus toxicus* and ciguatoxic fish flesh from the Caribbean Sea.

Biochemical property	Reference fish CTX	PPT-A (fat-soluble)	PPT-B (water-soluble)
Solubility			
Water	−	−/+	+
Methanol	+	+	+
Butanol	+	+	+
Acetone	+	−	−
Chloroform	+	+	−
Hexane	−	−	−
Column chromatography			
Silicic acid, column eluent (chloroform:methanol)	95:5	1:1	1:1
TLC system[1]:			
Toxic bands			
1	0.6-0.8	0.0-0.1[2]	0.0-0.1[2]
2	0.1-0.4	0.0-0.1[2]	
3	0.3-0.5	0.0-0.15[2]	
4	0.65-0.85	0.0-0.4	0.1-0.3

[1]Thin layer chromatography: Glass plates precoated with 1.0 mm silica gel (Brinkman SIL G-100 UV_{254} or Whatman PLK5F), activated 1 hour at 80°C, eluted after development with chloroform:methanol (3:1). Solvent systems: 1) chloroform:methanol (8:2), 2) benzene:butanol (75:25), 3) chloroform:methanol:6N ammonium hydroxide (90:9.5:0.5), and 4) chloroform:methanol:water (60:35:8).
[2]Bands re-eluted with methanol.

Discussion

Our toxicological results with field-collected *G. toxicus* are the first from a natural population in the Caribbean. Similar findings have been obtained from laboratory cultures of Caribbean *G. toxicus* (Tindall et al., 1984) with toxicity limited to the equivalents of our PPT-A and PPT-B. Methanolic extracts of cultures of *G. toxicus* from Puerto Rico were nontoxic (Tosteson et al., 1986), and unialgal cultures of the dinoflagellate from the Pacific Ocean often showed diminished toxicity (Bagnis et al., 1980), particularly under axenic conditions (Yasumoto et al., 1979a). To facilitate a comparison of our results (Tables 1 and 2) with published data obtained from wild Pacific material, a compendium of those reports is presented in Table 3. There seems to be general agreement with respect to the presence and chromatographic properties of a water-soluble toxin ("MTX", our PPT-B) and the absence of or only slight toxicity (Yasumoto et al., 1977b) in FLT-B and their equivalent acetone-soluble fraction. The symptomatology in mice is very similar, including the lack of convulsions before death (Bagnis et al., 1980), although they do not report measurements of body temperature. This apparent unanimity fails, however, concerning the lipid-soluble toxic component. Bagnis et al. (1980) obtained significant toxicity in two fat-soluble fractions: An acetone soluble "CTX" (our FLT-A) and an acetone precipitable "MTX" (our PPT-A). The latter was combined with the water-soluble toxin ("MTX", our PPT-B). Yasumoto et al. (1977b, 1979a) report a diethyl ether soluble fraction (our FLT-A and PPT-A combined), which was interpreted as corresponding to ciguatoxin ("CTX"). Secondary fat-soluble toxins were also found that were chromatographically similar to PPT-A (Yasumoto et al., 1976). We find lipid-soluble toxicity vir-

Table 3.—Yield and bioassay data in reports on extracts from ciguatoxic fish and wild *Gambierdiscus toxicus* from the Pacific Ocean.

Report	Sample	Extract designation and equivalent[1]	Average lethality in mice (cells × 10^4/20 g, range)	Chromatography[2] System	Chromatography[2] Toxic band or eluent
Bagnis et al., 1980	WGT: A-E	"CTX" (FLT-A)	4.8, 1.8-8.8	TLC-2 TLC-3 C.C.	0.2-0.6 0.2-0.4 9:1
		"MTX" (PPT-A and PPT-B combined)	0.7, 0.4-1.2	TLC-4 C.C.	0.1-0.3 4:6
	Reference fish CTX	"CTX" (CTX)		TLC-2 TLC-3 C.C.	0.0-0.3 0.25-0.4 9:1
Yasumoto et al., 1979a	Wild dinoflagellate (*Diplopsalis*, sp.) '75, '78	"CTX" (FLT-A and PPT-A combined)	31.4, 25.3-37.5	TLC-3 C.C.	0.5 9:1
Yasumoto et al., 1977b	*Diplosalis* sp. Fractions 2-4	"CTX" (FLT-A and PPT-A combined)	17.9, 8.1-25.4	TLC-2 TLC-3 C.C.	0.5-0.7 0.35-0.5 9:1
		"MTX" (PPT-B)	4.6, 0.8-8.4	C.C.	6:4 and 4:6
Yasumoto et al., 1980	Reference fish CTX	"CTX" (CTX)		TLC-2 TLC-3 C.C.	0.1-0.3 0.3-0.5 9:1
Yasumoto et al., 1976	Surgeonfish guts and contents	"MTX" (PPT-B) "Secondary toxins" fat soluble (PPT-A?)		TLC-4 C.C. C.C.	0.17-0.3 6:4 1:1

[1]Designations in reports: CTX = ciguatoxin, MTX = maitotoxin. See Figure 1 and Materials and Methods section for equivalents.
[2]TLC systems 1-4; silicic acid column chromatography (C.C.), eluent (chloroform:methanol); see Table 2.

tually limited to PPT-A, which behaves chromatographically as PPT-B, and no evidence of a toxin with chromatographic properties like reference Caribbean CTX. In fact, the chromatographic data cited to support the identity between Pacific *G. toxicus* "CTX" and reference Pacific fish "CTX" is less than conclusive (Table 3, compare particularly TLC-2 in Bagnis et al. (1980) and in Yasumoto et al. (1977b, 1980)). Interestingly, our reference Caribbean fish CTX seems remarkably similar to Pacific fish CTX in essentially the same chromatographic systems (compare Tables 2 and 3) and in HPLC (Higerd et al., 1986).

Pacific scientists offer much evidence, including food chain and gut-contents studies and surveys on the abundance of the dinoflagellate in ciguatera-endemic areas, to support the contention that *G. toxicus* is a biogenitor of toxins involved in ciguatera (previous references in Introduction, Table 3, and Bagnis et al., 1985; Yasumoto et al., 1977a, 1979b). We have also observed *G. toxicus* in the gut contents of herbivorous fish (unpubl.). And, even though the toxins of Caribbean *G. toxicus* appear to differ chemically from CTX, it would seem highly unlikely that PPT-A, PPT-B, and CTX could evoke essentially the same bioassay signs in mice, particularly the pronounced effect on body temperature (Sawyer, et al., 1984), and not be related. Considering all of the above from a circumtropical perspective, a substantially more uniform view of the ciguatera problem emerges. Unravelling the metabolic relationships among the toxins from *G. toxicus* and other dinoflagellates possibly involved (Yasumoto et al., 1980; Nakajima et al., 1981; Murakami et al., 1982; Bagnis et al., 1985; Tindall et al., 1984) as they are accumulated, transformed, and transferred through the foodweb represents a most promising but challenging aspect of understanding ciguatera.

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Toxin Production by *Gambierdiscus toxicus* Isolated from the Florida Keys

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ABSTRACT—The toxicities of six clonal Gambierdiscus toxicus *cultures collected concurrently from Knight Key, Fla., were compared with the toxicity of the Hawaiian* G. toxicus *strain, T39. LD_{50} values obtained using mouse bioassay demonstrated a hundredfold range in whole-cell toxicity. The Hawaiian and two Floridian strains had comparable mouse toxicity (LD_{50}) of about 2.5×10^4 cells/kg. Two additional groups of Floridian strains had toxicities of about 2×10^5 and >1 million cells/kg, respectively. Fractionation of methanol extracts by high-performance liquid chromatography suggests that toxins produced by different clones of* G. toxicus *are indistinguishable from each other but are more polar than fish toxin. Isolates of* Ostreopsis heptagona, *also isolated from Knight Key, had relatively low toxicities ($LD_{50} > 5 \times 10^6$ cells/kg).*

Introduction

Ciguatera is a tropical fish-borne disease in which the lipid-soluble neurotoxin, ciguatoxin, is believed to be transferred through the food chain and bioconcentrated primarily in carnivorous reef fish to levels toxic to humans. Since ecological research in the South Pacific implicated *Gambierdiscus toxicus* as the probable cause of ciguatera (Yasumoto et al., 1977), this dinoflagellate had been collected from the wild, grown unialgally, and extracted for toxins that have been analyzed principally by mouse bioassay.

Evidence that *G. toxicus* produces the fish-extracted ciguatoxin is based on reports in which either wild cells (Yasumoto et al., 1977, 1979; Bagnis et al., 1980; Shimizu et al., 1982) or cultured cells (Yasumoto et al., 1979; Tindall et al., 1984) were extracted and partitioned between solvents of different polarities. The biological and chemical nature, however, of the limited quantity of lipid-soluble dinoflagellate toxin obtained by these nonstringent extraction, partitioning, and purification techniques can only be conjecture. Cultured *G. toxicus* readily produce a toxin distinguished from ciguatoxin on the basis of higher molecular weight and lower polarity. This toxin has been tentatively identified as maitotoxin (Yasumoto et al., 1979), a toxin first isolated from the gut of surgeonfish, *Acanthurus* sp. (Yasumoto et al., 1976).

Unialgal cultures, initiated with up to 30 cells, are used in most studies on toxin production by cultured *G. toxicus*. The objectives of this study were to isolate and culture clonal *G. toxicus* collected concurrently from a single site in the Florida Keys, and compare the quantity and nature of the toxins produced. Toxicity, as measured in mice (LD_{50}), was determined using whole cells or nonfractionated extracted toxins, and the relative polarity of the extracted toxins was analyzed by high performance liquid chromatography (HPLC) (Higerd et al., 1986). The toxic characteristics of the Florida isolates are compared with a highly toxic clonal *G. toxicus* isolated in Hawaii (Sawyer et al., 1984).

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Materials and Methods

Benthic dinoflagellate samples were collected from the Florida Keys at a site previously involved in ecological studies on benthic dinoflagellates associated with ciguatera (Bomber, 1985). Samples were collected in December 1983, and in February, May, July, and December 1984, from Knight Key, which consistently had a high *G. toxicus* population. This site, station 3 of Bomber (1985), is located in Florida Bay, 0.8 km east of seven-mile bridge (lat. 42°44′ 20″N, long. 81°07′16″W). The area consists of an algal reef depauperate in coral with strong tidal currents and up to 95 percent cover by *Halimeda* spp. (Bomber, 1985). Pieces of macroalgae were collected in plastic jars with seawater, shaken, and the seawater filtered through a 250 μm Nitex[1] sieve. The filtrate was then refiltered through a 25 μm Nitex sieve and the retentate analyzed microscopically for *G. toxicus*. Samples with high populations of *G. toxicus* were suspended in 1 liter polycarbonate bottles containing 700 ml filtered (20 μm) seawater for shipment to the laboratory.

The medium used for isolation and growth of *G. toxicus* was Provasoli's ES enriched seawater medium as modified by J. West (McLachlan, 1973). Seawater was collected either at the Florida Keys

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

sampling site, or at two sites from 7 to 10 miles off Charleston, S.C., and filtered onsite through a 20 μm Nitex sieve into 10 to 20 liter polycarbonate carboys. Upon arrival at the laboratory, the water was refiltered through 0.45 μm cellulose nitrate membranes in a Nuclepore radial flow cell into sterile polycarbonate carboys and refrigerated until used to prepare media. The vitamin mixture (stored frozen) and enrichments were prepared in concentrated stocks, filter-sterilized and added aseptically to the seawater. The prepared medium was filter-sterilized through 0.2 μm cellulose nitrate membranes and used immediately or refrigerated.

Clonal cultures of *G. toxicus* and *Ostreopsis* sp. were established from single cells isolated with a micropipet, washed three or four times in sterile seawater, and inoculated into 16× 125 mm culture tubes containing 5 ml ES medium. *Ostreopsis* spp. occur with *G. toxicus* in the Florida sampling site (Bomber, 1985) and have been shown to be toxic by Nakajima et al. (1981). Growth was monitored with an inverted microscope, and if apparent within 30 days, the cultures were transferred to 25 ml mini-Fernbach flasks or 20 × 150 mm culture tubes containing 15 ml ES medium. After successful growth for 15-30 days, the cultures were placed in 250 ml polycarbonate Erlenmeyer flasks containing 100 ml ES medium. Stock cultures were maintained in 250 ml flasks and transferred to new medium every 30 days. These cultures were maintained at 27°C under an illumination of 30-50 $\mu E \cdot M^{-2} \cdot S^{-1}$ and a 16:8 hours light:dark cycle without aeration.

For toxin bioassay, 100 ml cultures were inoculated into 2.8 liter Fernbach flasks containing 1.5 liters ES medium. These cultures were grown for 30-45 days under incubation conditions described above. *Ostreopsis* clones were cultured for 10-21 days under the same conditions. Cell counts were determined in Palmer-Maloney chambers. To compare cell diameters with other reported *G. toxicus* isolates, cells were measured during their late log growth phase at 400× magnification with bright-field microscopy. Cells were harvested by filtering the culture medium through a 20 μm Nitex filter and resuspending the retentate in 30 percent aqueous methanol (v/v). This suspension was evaporated to dryness under a stream of nitrogen.

Toxicities of whole cells (LD_{50}) were determined by suspending the dried sample in phosphate buffered saline containing 5 percent Tween 80 and injecting 0.2 ml of appropriate cell concentrations intraperitoneally into female ICR mice weighing approximately 20 g (Kelly et al., 1986). In addition to determining whole cell toxicities, the seven *G. toxicus* clones were grown and harvested to obtain total cell densities greater than 1×10^6 cells. These samples were extracted in 80 percent aqueous methanol (v/v) for 48 hours at room temperature and bioassayed or fractionated by HPLC using a 50-100 percent methanol linear gradient. Each fraction was later assayed for toxicity by the mouse bioassay (Higerd et al., 1986).

Results and Discussion

Six *G. toxicus* clones were isolated concurrently from a dinoflagellate sample collected at Knight Key, 20 December 1983 (Bomber, 1985, station 3). The Hawaiian strain, T39, was hand-carried from Hawaii to the SEFC Charleston Laboratory. Five *Ostreopsis* clones represent a new species, *O. heptagona*, with cellular lengths >100 μm (Norris et al., 1985). *G. toxicus* clones were identified microscopically by their cellular shape and the characteristic "fishhook" apical pore slot (Adachi and Fukuyo, 1979; Taylor, 1979). Further taxonomic studies are in progress using a chloral hydrate-hydriodic acid staining method (Schmidt et al., 1978) and scanning electron microscopy.

Maximal cellular yields obtained for *G. toxicus* cultures grown for toxin bioassays were 1,000 cells/ml. Larger yields of 2,000-4,000 cells/ml have been reported for cultures of smaller *G. toxicus* (35-55 μm) (Bagnis et al., 1980; Carlson et al., 1984). On occasion, stored ES medium prepared with specific lots of natural seawater collected both from the Florida Keys and South Carolina coastal waters produced a precipitate that proved detrimental to growth and toxin production. This precipitate was prevented by eliminating the Tris buffer from ES medium.

Table 1.—Whole-cell toxicity of cultured *G. toxicus* clones.

Clone	Source	Cell dia. (μm)[1]	LD_{50} (cells/kg)
T39	Tern Island, HI	76	2.5×10^4 (4)[2]
Cd20	Knight Key, FL	79	3.5×10^4 (4)
Cd4	Knight Key, FL	81	4.5×10^4 (3)
Cd8	Knight Key, FL	94	2.5×10^5 (2)
Cd10	Knight Key, FL	81	3.0×10^5 (2)
Cd9	Knight Key, FL	86	$>2.3 \times 10^6$ (2)
Cd13	Knight Key, FL	77	$>2.5 \times 10^6$ (2)

[1]Cellular measurements were obtained by measuring live individuals at 400× with bright-field microscopy (n = 30).
[2]Bioassay analyses (n).

Five *O. heptagona* clones isolated from Knight Key, prepared and bioassayed using the same procedure described for *G. toxicus*, had relatively low toxicity ($LD_{50} > 5 \times 10^6$ cells/kg. Mouse mortalities were observed at injections of 5×10^6 cells/kg, but higher dosages were not assayed so a definitive LD_{50} could not be calculated. Although *O. siamensis* and *O. ovata* were shown to be toxic by Nakajima et al. (1981), their toxicities were also $> 5 \times 10^6$ cells/kg. Besada et al. (1982) reported no toxicity in *Ostreopsis* cultures isolated from the Caribbean Sea.

The LD_{50} values using whole cells of *G. toxicus* are shown in Table 1. The Hawaiian clone, T39, was similar in toxicity to Cd20 and Cd4 from the Florida Keys and to T39 cultures grown in Hawaii. Florida Key clones Cd8 and Cd10 were 10 times less toxic, while the toxicities of Florida Key clones Cd9 and Cd13 were just evident with more than 2×10^6 cells/kg. As with *O. heptagona*, mortalities were observed, but higher dosages were not assayed so a definitive LD_{50} could not be calculated. LD_{50} values for nonfractionated methanol extracts of two Cd20 cultures were 1×10^5 cells/kg, or about 70 percent less toxic, than whole cells.

This is the first report of comparative toxicities using clonal cultures of *G. toxicus* isolated concurrently from a single site. Two unialgal cultures isolated from the Florida Keys have been reported in a previous study (Bergmann and Alam,

Table 2.—Relative HPLC elution times for toxic fractions of extracted *G. toxicus*.

Source of extracted material	^1R_t
G. toxicus (Hawaii) T-39	2.2
G. toxicus (Hawaii) T-39	2.1
G. toxicus (Florida) Cd-4	2.8
G. toxicus (Florida) Cd-4	2.6
G. toxicus (Florida) Cd-10	2.6
G. toxicus (Florida) Cd-20	2.7
Fish (St. Thomas, U.S.V.I.)	4.0

[1]Ratio of toxic activity elution time relative to elution time for phenol standard, where phenol = 1.

1981) and these cultures had toxicities in mice of 3.1×10^5 cells/kg and 1.2×10^5 cells/kg.

To compare polarities of dinoflagellate toxins, the ratio between the toxic fraction elution times from a HPLC column for extracted dinoflagellates and a phenol standard was calculated. Results were expressed as a relative retention time (R_t), with phenol equivalent to 1.00 (Table 2). Only a single toxic component was detected in each of the four *G. toxicus* cultures and they exhibited similar R_t values. In contrast, the dinoflagellate toxins were far more polar than the fish toxin, which eluted much later in the linear methanol gradient. Since toxic fractions were detected by mouse bioassay, this technique may have missed toxic fractions since LD_{50} values $\geq 1.0 \times 10^6$ cell/kg were not assayed. Cells extracted from the remaining three *G. toxicus* samples did not provide enough toxic activity to be detected with this procedure. The chromatographic details of this HPLC technique can be found in an accompanying conference paper (Higerd et al., 1986).

This study demonstrated a hundredfold variation in toxicity of clones isolated concurrently, which would suggest that toxin production may vary in natural populations of *G. toxicus*. Unialgal cultures, initially containing several competing clones, might produce variable toxin profiles until a single clone became dominant and the other clones were lost through transfer dilution. Cultural parameters can also influence the relative toxicity of *G. toxicus* (Bergmann and Alam, 1981) since maitotoxin, the primary and most potent *G. toxicus* toxin, is produced late in the growth phase (Yasumoto, et al., 1979). Insufficient data in other studies and the lack of a standardized mouse bioassay made it difficult to compare quantitatively the levels of *G. toxicus* toxicity reported in this study with previous investigations. However, approximate toxicities reported for cultured *G. toxicus* range from 6×10^3 cells/kg (Yasumoto et al., 1979) to 1×10^6 cells/kg (Tindall et al., 1984). Toxicity quantitation presently is limited by the nonspecific nature and lack of precision and accuracy of the mouse bioassay.

Toxins extracted with aqueous methanol were 70 percent less toxic than whole cells as measured by mouse bioassay, indicating that the toxic moieties of extracted nonfractionated toxins and whole cells may differ or that extraction efficiency requires improvement. Tindall et al. (1984) reported ciguatoxin, one ciguatoxin derivative, and maitotoxin present in fractionated extracts of cultured unialgal *G. toxicus*. Their limited data, however, could not exclude the possibility of multiple toxins derived from carryover of maitotoxin during the extraction and separation procedures as observed by Yasumoto et al. (1979) or interchangeable toxin forms like that reported for purified ciguatoxin (Nukina et al., 1984). The HPLC system used in the current study permitted distinct separation of several toxins, but because of limited amounts of extracted material, toxins present in minor quantities may have gone undetected.

There is a need to identify and quantitate toxins present in cultured benthic dinoflagellates associated with ciguatera. An extremely valuable analytical method for determing total toxin profiles has been developed for paralytic shellfish poisoning (PSP) toxins (Sullivan and Wekell, 1984; Sullivan et al., 1985). This HPLC analysis uses derivatives of PSP toxins to fluorometrically detect toxic fractions separated in an HPLC column at concentrations four times more sensitive than mouse bioassays. A technique similar to the HPLC procedure for PSP toxins has been developed for detecting toxin extracted from *G. toxicus* (Sick et al., 1986). Such a technique may be capable of quantitating and comparing the chemical nature of toxins from clonal *G. toxicus* cultures and determining the effects that cultural parameters have on toxin profiles, similar to a study reported for PSP toxins (Boyer et al., 1985).

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Comparative Toxicity of *Gambierdiscus toxicus*, *Ostreopsis* cf. *lenticularis*, and Associated Microflora

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Introduction

The benthic dinoflagellate genera *Gambierdiscus* and *Ostreopsis* are commonly found in tropical marine environments (Besada et al., 1982; Steidinger, 1983). Regions in which they are found are often characterized by frequent outbreaks of ciguatera fish poisoning (Besada et al., 1982). In the coastal waters of southwest Puerto Rico, these dinoflagellates are abundant (Ballantine et al., 1986). Tindall et al. (1984) has speculated that a variety of dinoflagellates contribute to ciguatera fish poisoning. In this paper, we describe the comparative toxicity of methanolic extracts of *G. toxicus* and *O. lenticularis* grown in clonal culture, and the possible influence of their associated microflora.

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Materials and Methods

Microbial Cultures and Extraction Procedures

Gambierdiscus toxicus and *O. lenticularis* were collected from an inshore coral reef located 1 km offshore from La Parguera on the southwest coast of Puerto Rico. Clonal cultures of both dinoflagellates were initiated and subsequently maintained in both F/2 (Guillard and Ryther, 1962) and ES media (Provasoli, 1968). Since February 1983, we have initiated five clonal cultures of *G. toxicus* and seven of *O. lenticularis*. Batch cultures were grown at 26°C in a light regime of 16:8 hours (light:dark) at a flux of 40 microeinsteins/m^2/second. During the study, on the basis of preliminary results obtained using a cross gradient culture apparatus (Edwards, 1970), the light flux for the cultures of *O. lenticularis* was increased to 130 microeinsteins/m^2/second. Cultures of *Gambierdiscus* reached maximum population densities in 3-4 weeks, while *Ostreopsis* required 2 weeks. Volumes of 3-4 liters were harvested weekly with a total yield of 1-3.5 × 10^6 cells of each dinoflagellate. Cells were harvested by filtration (Minitan System, Millipore[1]) and screening (35 μm mesh). Cell aliquots were briefly rinsed with distilled water and sonicated in redistilled methanol. Extracts (final volumes of about 100 ml) were allowed to remain at laboratory temperature (22°C) for 48-96 hours. Extract suspensions were then filtered (Whatman, #1) and the filtrate solvent removed by flash evaporation (Buchi, Rotavapor). The resulting residues were taken to dryness under nitrogen and stored in a vacuum desiccator for later toxicity studies.

The microflora associated with the dinoflagellates were periodically evaluated by streaking aliquots from *Ostreopsis* and *Gambierdiscus* cultures on solid media made from an enriched seawater solution consisting of 2 g peptone, 2 g trypticase, 1 g yeast extract, and 1 mcg vitamin B_{12} per liter of seawater. On several occasions, the microflora associated with aliquots of cell free media and cell concentrates were evaluated as described above. Bacterial isolates were purified, maintained in laboratory culture using the cited medium

ABSTRACT—Benthic, epiphytic dinoflagellates, Gambierdiscus toxicus, *and an ecological associated dinoflagellate,* Ostreopsis *cf.* lenticularis, *were isolated from macroalgal hosts at a shallow inshore reef habitat off the southwest coast of Puerto Rico. These dinoflagellates were isolated into clonal cultures and are maintained in laboratory culture employing enriched seawater media. Four bacterial strains have been isolated from cultures of* G. toxicus *and* O. lenticularis. *One strain belonged to the family Pseudomonadaceae, two were Vibrionaceae, and the fourth was from the family Nocardiaceae. Three strains could be recovered from culture media in which the dinoflagellates were grown, while* Nocardia *sp. was associated only with the dinoflagellate cells themselves. Methanolic extracts of Puerto Rican* G. toxicus *were not toxic when inoculated (i.p.) in mice, while similar extracts of* O. lenticularis *were toxic.* LD_{50} *values obtained for these extracts ranged from 6.5 to 72.5 mg/kg mice. The highly variable toxicity of* O. lenticularis *extracts appeared to be correlated with the abundance of the* Nocardia *strain recovered from disrupted cells of this dinoflagellate.*

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

and Zobell 2216E medium, and later identified to the genus level (Colwell, 1984; Buchanan and Gibbons, 1974; Koneman et al., 1983; Zobell, 1944).

Routinely at the end of each culture growth period, bacteria directly associated with *Ostreopsis* cells were monitored. Aliquots of cells from each culture were disrupted by sonication, and serial dilutions of the resulting particulate suspensions were inoculated onto bacterial media. The number of bacteria associated with the *Ostreopsis* cells was quantitated (spread plate technique) and cellular ratios calculated. Microbes isolated from dinoflagellate cultures were also grown in batch culture, harvested, and extracted with methanol in the same manner as the dinoflagellate cells described above.

Toxicity Assay

Initial screening and LD_{50} determinations were done employing ICR female mice weighing about 20 g each. Animals were maintained on Wayne Laboratory Animal diets (Lab-Glox) and water, ad libitum. Known quantities of dried extracts to be tested were suspended in 0.15 M phosphate buffer solution (PBS) containing 5 percent Tween 80. Inocula of 0.2 ml were administered by intraperitoneal (i.p.) injection. Each extract was tested at from 4 to 8 concentrations, decreasing geometrically from the highest level tested (5-6 mg/20 g mouse). Three or four mice were inoculated at each extract concentration. Extracts that showed no toxicity at the highest level tested were considered nontoxic. Control animals received injections of equal volumes of the Tween PBS media. Mice were observed for periods of 48 hours. LD_{50} values were calculated according to the method of Weil (1952).

Results and Discussion

Methanolic extracts of Puerto Rican *G. toxicus* were not toxic when inoculated i.p. in mice, while similar extracts of *O. lenticularis* were toxic. To our knowledge, this is the first reported toxicity for this dinoflagellate species. Toxicity has been reported for two other species of *Ostreopsis* (Nakajima et al., 1981). The LD_{50} values obtained for the *O. lenticularis* extracts varied considerably, ranging from 6.5 to 72.5 mg/kg mice. These LD_{50} values are comparable to those reported for other tropical benthic dinoflagellates (Tindall et al., 1984). Animals inoculated with toxic extracts of *O. lenticularis* displayed similar symptoms and appearance to mice injected with ciguateric fish toxins (Hoffman et al., 1983).

Four bacterial strains were isolated from *G. toxicus* and *O. lenticularis* cultures. The distribution of these strains is given in Table 1. *Aeromonas* sp. and *Vibrio* sp. were routinely recovered from cell-free media of *G. toxicus* cultures, while *Pseudomonas* sp. was regularly found in the cell-free media of *O. lenticularis*. A distinctive bacteria forming crusty aggregates on the surfaces of both solid (agar) and aqueous media, identified as *Nocardia* sp., was prominently found in preparations of *O. lenticularis* cells. This bacterial strain was only detectable in *G. toxicus* cells of the first clone isolated. *Nocardia* sp. has not been found in repeated sampling of the four subsequently isolated clones of *G. toxicus*. Initial tests indicated that the methanolic extract of *Nocardia* sp. was toxic to mice at dosages between 6 and 9 mg/animal; however, subsequent repeated testing revealed that the methanolic extracts of this bacterial isolate were not toxic to mice. The other bacterial strains associated with the dinoflagellate cultures also failed to show toxicity. The LD_{50} of *O. lenticularis* extracts was inversely related to the *Nocardia*/*Ostreopsis* cell ratio (Table 2). Thus, while *Nocardia* sp. did not remain toxic when repeatedly grown in laboratory culture, its relative abundance in *O. lenticularis* cells may play a role in determining the toxicity of this dinoflagellate. The genus *Nocardia* is characterized by the presence of pathogenic species (Buchanan and Gibbons, 1974). Mitogenic and adjuvant active materials have additionally been extracted from a number of *Nocardia* species (Ciorbaru et al., 1974).

Elevated light intensities stimulated the growth rate of *Ostreopsis* cultures. The steady state number of *Nocardia* cells associated with *Ostreopsis* in the light stimulated *O. lenticularis* cultures significantly decreased (Table 3). In *O. lenticularis* batch cultures, there was considerable variation in growth rate

Table 1.—Dinoflagellates and their associated bacterial strains.[1]

G. toxicus		
Clone I	Clone II-IV	*O. lenticularis*
GT5: *Nocardia* sp. (slight)	None	06: *Nocardia* sp. (heavy)
GT4: *Aeromonas* sp.	GT4: *Aeromonas* sp.	07: *Pseudomonas* sp.
GT16: *Vibrio* sp.	GT16: *Vibrio* sp.	

[1]GT4 and 16 belong to the family Vibrionaceae, 06 is Nocardiaceae, and 07 is Pseudomonadaceae.

Table 2.—*Ostreopsis lenticularis* toxicity and associated bacterial densities.

Preparation[1]	Associated bacteria (per dinoflagellate cell)	[2]LD_{50}
6001 (24 Oct.)	9	[3]0
6015 (20 Nov., 5 Dec.)	74	41
6002 (8 Nov.)	298	6.5

[1]Average number of extracted cells: 1.457 ± 0.068(SE) × 10^6.
[2]Lethal dose 50 percent given in mg/kg mice.
[3]No animals died within the experimental period of 48 hours.

Table 3.—*Ostreopsis lenticularis*: Light flux, growth, and associated bacterial densities.

Light flux[1]	*Nocardia* sp./ *O. lenticularis*	Dinoflagellate doubling time (da)	Dinoflagellate cells harvested ($\times 10^6$)
130	2	4.0	4.82
40	110	6.1	1.27

[1]Microeinsteins/m^2/second.

Table 4.—*Ostreopsis lenticularis*: Growth and associated bacteria densities.

Final dinoflagellate concentration (cells/ml)	Bacteria/ dinoflagellate	Doubling time (da)
686	7.3	11.0
1,238	2.6	6.6
645	10.2	11.9

among the culture flasks that constituted a given batch. This effect is illustrated in Table 4. The slower growing cultures were characterized by significantly greater *Nocardia*/*Ostreopsis* cell ratios than that seen in more rapidly growing cultures. It is of interest to note that the cell densities of *Ostreopsis* growing epiphytically on *Dictyota* spp. in the field also showed great variability at a given sampling site (Ballantine et al., 1986). The decrease in *Nocardia*/*Ostreopsis* cell ratios in more rapidly growing dinoflagellate cultures may have been due to the fact that the growth of the associated *Nocardia* populations was not keeping pace with increased dinoflagellate growth in laboratory culture conditions.

A better understanding of the precise relationship between increased *Ostreopsis* growth, *Nocardia*/*Ostreopsis* cell ratios, and *Ostreopsis* toxicity awaits further data and analyses. The data presented here suggest that *O. lenticularis* and its associated microflora may be a primary ciguateric vector in the coastal waters of southwest Puerto Rico.

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Economic Aspects of the Japanese Kamaboko Industry

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Introduction

The newest and most promising seafood technology impacting U.S. markets in recent years is an ancient Japanese fish paste process which yields a final product called kamaboko, a fish protein gel which is flavored and formed to suit the tastes and preferences of consumers. Many varieties of kamaboko have been developed for the Japanese market (Table 1), and since 1976, several forms of kamaboko have been exported from Japan to the United States including: A lobster tail analog, scallop analog, shrimp analog, and crab analog. In this paper we discuss various economic aspects of trade, marketing, and production which affect the kamaboko industry of Japan and impact the U.S. market for Japanese kamaboko.

Kamaboko Manufacture

Kamaboko is manufactured from minced/washed fish which is ground with sugar and other flavorings, shaped, heated, and cooled to form a final product. To produce minced/washed fish from fresh fish, the fish is headed and gutted, deboned, and washed. The minced/washed fish may go directly into kamaboko production (fresh-fish method) or it may be frozen and used later. To prevent deterioration of the flesh in frozen form, cryoprotectants (sugars) are added to the minced fish. This process yields an intermediate product called surimi, the history and manufacture of which are discussed in the following section.

To produce kamaboko from surimi (surimi method), the surimi must first be thawed. After thawing, the surimi is really no different than the minced/washed fish used in the fresh fish method except that it already contains cryoprotectants which are required to manufacture kamaboko. The decision to use either surimi or fresh fish as a base raw material for the manufacture of kamaboko is solely dependent upon geographic and economic considerations. The quality of the final product is not altered by the method employed. The resilient texture of kamaboko, referred to as "ashi" by the Japanese, is a major factor in determining product quality. The ashi is determined by the species, freshness, and size of the fish used, fishing method, and fishing season (Suzuki, 1981).

The fresh-fish method is limited to a scale of production directly associated with raw fish availability, while the surimi method enables large-scale production associated with the ability to store large quantities of frozen surimi. Where the fresh-fish method is job-oriented, the surimi method is process- and flow-oriented which, in most cases, translates to a more efficient use of capital through larger outputs using similar capital requirements, i.e., cost of plant, cost of machinery, and fixed overhead costs. The surimi method, however, has additional costs associated with the freezing, holding, and handling of the intermediate product. If these additional costs are greater than the efficiency gains of the process- and flow-operation, it may be economical to forego the surimi process in favor of producing kamaboko directly from fresh fish. In 1984, about 38 percent of Japanese kamaboko was produced from fresh fish[1].

Japanese domestic production of kamaboko totaled 1,020,028 metric tons (t) in 1984 (Table 2). Total production increased 11.7 percent from the 1980 level. Exports of kamaboko increased fifteenfold, growing from 0.25 percent of total production in 1980 to 3.5 percent in 1984. U.S. imports, as a percentage of total Japanese exports, grew from 44.7 percent in 1981 to 82.1 percent in 1984.

Table 1.—Variations of kamaboko (Suzuki, 1981).

Item	Variation[1]
By heating method:	Steamed
	Steamed and broiled
	Broiled
	Broiled (hampen)
	Fried (tempura, satsuma age)
By shape:	Piled on a thin wooden slab (itatsuki)
	Tubular (chikuwa)
	Ball, bar, or square (age)
	Leaf (susa)
	Needle (soba)
	Rolled (datemaki)
	Chipped (kezuri)

[1]Japanese name in parentheses.

Surimi Manufacture

The Japanese have fished for walleye pollock, *Theragra chalcogramma*, in the waters between Japan and Alaska for many years. The primary value of the fish had traditionally been attributed to its roe, considered a delicacy in Japan. Although the flesh is also valued, the quality of fillets processed from the fish-

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[1]Assumptions are surimi produced in year *t* was processed into kamaboko in year *t*, and the average surimi-based kamaboko product contained 60 percent surimi and had a yield of 1.67, kamaboko to surimi.

ery was low due to deterioration of the flesh even after freezing.

To prevent deterioration, the fish were washed, minced, and mixed with sugar and polyphosphate to produce mu-en (salt free) surimi. Ka-en (salted) surimi was also produced by adding salt to the mixture, replacing the polyphosphate. K. Nishiya has been credited with the discovery of the process and preliminary research regarding methodology (Nishiya et al., 1961), and by 1964 large-scale onshore production of pollock surimi was realized[2].

Surimi has developed its own market as an input for the production of kamaboko, and a number of firms have appeared in the industry that produce only surimi which is sold on the open market and further produced into kamaboko by another set of firms. If the cost per unit of surimi is less than the cost of procuring and mincing a unit of fish, it would be favorable to purchase surimi and forego the mincing and washing operations.

A fairly strong analogy can be drawn between surimi and frozen fish blocks. Both participate in factor markets in that these goods are not directly consumed; surimi and fish blocks are the major cost and quantity components of kamaboko and "fish sticks and portions," respectively; the demand for surimi and frozen blocks is derived directly from the demand for their final products; and the supply of each input factor is a function of the biological abundance of species utilized and fishing effort directed toward those species.

In 1964 there were 39 surimi factories located in Japan which produced a relatively low-grade surimi. The low quality was attributable to the length of time required between catch (offshore) and process (onshore). To increase the quality of pollock surimi, factory vessels were introduced in 1965. The number of factory vessels as a percentage of total factories grew from 4.7 in 1965 to 26.5 in 1982 (Table 3). Much of this increase resulted from the decline in the number of onshore facilities and not from the growth in the number of offshore factory ships.

Average annual production of factory vessels from 1971 to 1983 was much larger than that of onshore facilities, 189,368 t/year and 149,213 t/year, respectively. Supply from onshore facilities for the same period totaled 1,939,769 t while the supply from factory vessels totaled 2,461,781 t.

Although the number of onshore facilities had generally declined over the period, the average annual production per plant had grown dramatically. In 1972, there were 105 plants producing an average of 1,536 t of surimi, compared with 61 plants in 1983 producing an average of 2,623 t. This reflects an increase in production per plant of 71 percent. About 90 percent of all surimi is processed from walleye pollock, but Atka mackerel, *Pleurogrammus monopterygius*; croaker (Sciaenidae); jack mackerel, *Trachurus symmetricus*; and lizardfish, *Synodus lucioceps*, are also used (Hotta, 1982).

Japanese domestic production of surimi totaled 384,275 t in 1984 (Table 4), an increase of 5.5 percent from the 1980 level. Domestic firms utilized 99.3 percent of the 1984 production. The Japanese exported 2,580 t in 1984, about 3.5 times the 1980 level. The United States was the major importer of surimi from 1980 through 1984.

Surimi and Kamaboko Production Ouside of Japan

The limited production of surimi and kamaboko outside Japan through 1984

Table 2.—Kamaboko production and export from Japan in metric tons[1], 1980-84.

Nation and year	Exports (t)	Percent change from 1980	Percent of total export	Percent change per annum
United States				
1980	1,482		65.8	
1981	2,603	+75.6	44.7	+75.6
1982	7,320	+393.9	59.2	+181.2
1983	14,982	+910.9	63.8	+104.7
1984	29,387	+1882.9	82.1	+96.1
England				
1980	9		0.3	
1981	15	+66.7	0.3	+66.7
1982	376	+3177.8	3.0	+2406.7
1983	2,246	+23955.5	9.6	+497.3
1984	1,769	+18655.5	4.9	−21.2
Australia				
1980	67		3.0	
1981	2,191	+3170.1	37.6	+3170.1
1982	2,915	+4250.7	23.6	+33.0
1983	2,846	+4147.8	12.1	−2.4
1984	1,883	+2710.4	5.3	−33.8
Others				
1980	695		30.8	
1981	1,016	+46.2	17.4	+46.2
1982	1,753	+152.2	14.2	+72.5
1983	3,398	+388.9	14.5	+93.8
1984	4,933	+609.8	13.8	+45.2
Total				
1980	2,253			
1981	5,825	+158.5		+158.5
1982	12,364	+448.8		+112.3
1983	23,472	+941.8		+89.8
1984	35,791	+1488.6		+52.5
	Kamaboko production			
Japan				
1980	913,186			
1981	948,882	+3.9		+3.9
1982	960,900	+5.2		+1.3
1983	996,171	+9.1		+3.6
1984	1,020,028	+11.7		+2.3

[1]Data furnished by Hirochika Katayama, Japan-U.S. Trade Office, Washington, D.C.

Table 3.—Surimi production, in metric tons, from walleye pollock in Japan[1].

Year	Onshore production	No. of plants (n=24)	Offshore production	Factory ships (n=19)	Total production
1960	250	4			250
1961	2,500	9			2,500
1962	4,500	14			4,500
1963	9,282	28			9,282
1964	18,060	39			18,060
1965	23,639	41	8,184	2	31,823
1966	29,913	54	13,034	5	42,947
1967	44,869	58	39,220	7	84,089
1968	69,635	121	75,837	9	145,472
1969	92,718	110	103,610	14	196,328
1970	118,522	108	142,802	16	261,324
1971	137,848	110	183,534	20	321,382
1972	161,308	105	193,548	21	354,856
1973	159,146	103	223,598	23	382,744
1974	152,829	100	195,297	23	348,126
1975	169,036	97	191,730	22	360,766
1976	197,559	125	187,807	22	385,366
1977	193,123	111	168,823	22	361,946
1978	132,432	89	183,012	19	315,444
1979	114,426	85	180,402	20	294,828
1980	105,669	76	183,232	20	288,901
1981	114,393	66	192,264	22	306,657
1982	142,000	61	198,534	22	340,535
1983	160,000	61	180,000	22	340,000
Avg. 1971-83[2]	149,213		189,368		

[1]From a report to the Overseas Fishery Cooperative of Japan by the Deep Sea Trawlers Association, 1983, 103 p., unpubl., copy on file at SEFC Charleston Laboratory, Charleston, S.C.
[2]These averages are significantly different at the 0.01 probability level with $t = -4.61$ and d.f. = 16.8.

[2]From a report to the Overseas Fishery Cooperative of Japan by the Japan Deep Sea Trawlers Association, 1983, 103 p., unpubl. A copy of this report is on file at the SEFC Charleston Laboratory, NMFS, NOAA, P.O. Box 12607, Charleston, SC, 29412-0607.

came, for the most part, from joint ventures established by Japanese companies. The fish used for surimi production are those that produce a high-value product for the Japanese market. Croaker has been the most popular species used due to its high quality ashi. Participating countries include, but are not limited to, Thailand, Taiwan, and the United States. Kamaboko production in these countries relies for the most part on pollock surimi imported from Japan. To achieve economic benefits from this method of trade in surimi, i.e., croaker surimi imported to Japan and pollock surimi exported from Japan, the Japanese must have a comparative advantage in the production of pollock surimi and a comparative disadvantage in the production of croaker surimi.

A simplistic estimate of comparative advantage/disadvantage may be made by examining relative prices. In this case, the following condition must hold:

$$\frac{Pollock\ surimi_{JP}}{Croaker\ surimi_{JP}} < \frac{Pollock\ surimi_{TPP}}{Croaker\ surimi_{TPP}}$$

where:

JP = Japanese price and
TPP = Trading partner's price.

These price ratios are referred to as the terms of trade and are one factor in determining both the levels and the direction that trade occurs.

A New Zealand company manufactured surimi from barracouta, *Thyrsites atun*, on an experimental basis, and found that the process was not economically feasible due to a yield from round barracouta of 17-18 percent for surimi compared with a yield of 33 percent for fish blocks. The U.S.S.R. has unsuccessfully attempted to produce surimi from walleye pollock for the Japanese market. The reason most commonly given for their failure is "poor quality product" (Hotta, 1982).

Table 4.—Surimi production and export from Japan in metric tons[1], 1980-84.

Nation and year	Exports (t)	Percent change from 1980	Percent of total export	Percent change per annum
United States				
1980	703		99.2	
1981	819	+16.5	88.6	+16.5
1982	1,114	+58.5	87.3	+36.0
1983	1,708	+143.0	87.0	+53.3
1984	2,306	+228.0	89.4	+35.0
Europe				
1980	6		0.8	
1981	7	+16.7	1.0	+16.7
1982	5	−16.7	0.7	−28.6
1983	4	−33.0	0.6	−20.0
1984	6	0	0.8	+50.0
Australia				
1980	0		0.0	
1981	5		0.7	
1982	0		0.0	
1983	0		0.0	
1984	212		8.4	
Others				
1980	0		0.0	
1981	97		10.5	
1982	157	+61.8	12.3	+61.8
1983	251	+340.4	12.9	+59.9
1984	56	−42.3	2.2	−77.7
Total				
1980	709			
1981	928	+30.9		+30.9
1982	1,276	+80.0		+37.5
1983	1,963	+176.9		+53.8
1984	2,580	+263.9		+31.4
	Surimi production			
Japan				
1980	364,246			
1981	354,922	−2.6		−2.6
1982	373,049	2.4		5.4
1983	379,873	4.3		1.8
1984	384,275	5.5		1.2

[1]Data were furnished by Hirochika Katayama, Japan-U.S. Trade Office, Washington, D.C.

Industry Structure

The Japanese offshore surimi industry is controlled by five firms, three of which produce 90 percent of the total product (Hotta, 1982). These firms sell top-grade surimi to a large number of kamaboko producers in Japan and a limited number of kamaboko producers outside of Japan. The majority of firms outside Japan are subsidiaries of Japanese firms; therefore, a limited amount of money in the form of labor cost, overhead cost, taxes, and cost of plant actually leaves Japan. This is especially true since these plants use Japanese machinery and equipment in their operations.

The Economic Impact of EEZ Legislation

The Japanese have lost access to a major portion of Alaska's walleye pollock fishery in the Bering Sea with the advent of the 200-mile U.S. Exclusive Economic Zone (EEZ), but the resource is still available through joint ventures with U.S. fishermen. It is likely that these arrangements have translated into higher fish raw material costs for Japanese offshore producers. It is not clear, however, as to the extent the United States benefits from the EEZ legislation. Given the structure of the Japanese offshore industry, few firms supplying a large number of buyers, it is likely that surimi producers are passing these higher costs on to kamaboko producers, who, in turn, may pass at least a portion on to consumers both in Japan and in the United States. Therefore, in a strict welfare context, the American consumer may be forced to pay a higher price for Japanese kamaboko, reducing the sum of the benefits of the EEZ to the United States, i.e., American fishermen gain and American consumers of Japanese-produced kamaboko lose, but since the major market for kamaboko is Japan, U.S. society, as a whole, should gain from these arrangements.

Substitution of Crab Analog for King Crab Meat

The crab analog form of kamaboko has gained wide acceptance in the United States. It first appeared on the market in 1978 in small quantities in the form of imitation[3] crablegs, and more recently lump and chunk forms of imitation crabmeat have been introduced.

The export of crab analog as a percentage of total Japanese kamaboko export increased from 64.1 percent in 1980 to 98.9 percent in 1984. In 1984 the United States imported 29,188 t which accounted for 82.4 percent of Japanese exports (Table 5). Although it is apparent that the Japanese have been very successful in marketing crab analog, it is not clear why. Many people postulate that an increase in king crab, *Paralithodes camtschatika*, prices, due to drastically reduced landings, encouraged consumers to purchase the crab analog as a lower priced replacement.

To investigate this assumption, a price function to estimate the demand for king crab meat at the wholesale level was specified. In the absence of price data (due to the newness of imported crab analog), a dummy variable was incor-

[3]The term "imitation" is descriptive of the product. The exact nomenclature as specified by the FDA has not been resolved.

porated into the regression which reflects the effect on king crab prices that was not predicted by historical demand and supply patterns in the years 1981-84.

A structural change in the marketing of crab meat which concentrated on the restaurant trade was coincidental with the large increases in demand for crab analog in the period 1981-84 (Table 5). Therefore, we assume that the dummy variable is capturing the price effect of the summation of the new marketing initiatives for king crab and the increased demand for the Japanese kamaboko product. The marketing initiatives have a positive effect on price through increased demand for crab meat which causes the dummy variable to have a positive sign. Conversely, the replacement factor of crab analog for crab causes a decreased demand for crab meat causing the dummy variable to have a negative sign.

It is assumed that crab meat supplies are a function of abundance and thereby are assumed to be predetermined. It is also assumed that yields of crab meat from the whole crab have remained unchanged over the period. Prices and income are deflated by the GNP-implicit price deflator to preclude inflationary effects. The equation utilizes 14 years of annualized data, 1971-84, inclusive, and is estimated using the ordinary least squares technique.

Table 5.—Crab analog export from Japan in metric tons[1], 1980-84.

Nation and year	Exports (t)	Percent change from 1980	Percent of total export	Percent change per annum
United States				
1980	1,254		86.8	
1981	2,228	+77.7	55.2	+77.7
1982	6,749	+438.2	72.3	+202.9
1983	13,823	+1102.3	73.4	+104.8
1984	29,188	+2327.6	82.4	+111.2
Europe				
1980	0		0.0	
1981	27		0.6	
1982	597	+2211.1	6.4	+2211.1
1983	2,752	+10092.5	14.6	+361.0
1984	2,632	+9648.1	7.4	−4.4
Australia				
1980	120		8.3	
1981	1,734	+1445.0	43.0	+1445.0
1982	1,750	+1458.3	18.8	+0.9
1983	1,581	+1317.5	8.4	−9.7
1984	1,791	+1492.5	5.1	+13.2
Others				
1980	70		4.8	
1981	44	−37.1	1.1	−37.1
1982	234	+234.3	2.5	+431.8
1983	673	+861.4	3.6	+187.5
1984	1,562	+2131.0	4.4	+132.1
Total				
1980	1,444		64.1	
1981	4,033	+179.3	69.2	+179.3
1982	9,330	+546.1	75.5	+131.3
1983	18,829	+1203.9	80.2	+101.8
1984	35,413	+2352.4	98.9	+88.1

[1]Data were furnished by Hirochika Katayama, Japan-U.S. Trade Office, Washington, D.C.

Functional Form Equation

$$PK = f(-Q, +PSN, +Y, \pm D)$$

where

- PK = average annual wholesale price of king crab meat in real cents per pound at Fulton Fish Market, N.Y., in year t (1972 = 1.0),
- Q = quantity demanded in millions of pounds in year t,
- PSN = average annual wholesale price of snow crab, *Chionocetes* sp., meat in real cents per pound at Fulton Market, N.Y., in year t (1972 = 1.0),
- Y = per capita income in year t, and
- D = dummy variable, where 1971-80 = 0 and 1981-84 = 1.

Estimated Equation[4]

$$PK = -427 - \underset{(-1.94)}{1.40^{*}}\,Q + \underset{(1.55)}{0.738^{*}}\,PSN + \underset{(2.11)}{0.189^{*}}\,Y + \underset{(2.65)}{183^{*}}\,D$$

$$R^2 = 82.6 \quad F_{(4,9)} = 16.39^{*} \quad d = 1.97$$

Interpretation

The price flexibility of demand for king crab meat is measured at −0.215, which means a 1 percent increase in quantity demand will decrease price by 0.215 percent. The cross-price flexibility of snow crab meat for king crab meat is measured at 0.410, meaning a 1 percent increase in the price of snow crab meat will cause an increase in the price of king crab meat of 0.410 percent. The price flexibility of income for king crab meat is measured at 1.560, meaning that a 1 percent increase in per capita income will cause the price of king crab meat to increase 1.560 percent. All measurements were taken at the centroid, or center point of the demand curve.

The deflated price of king crab meat rose from $5.40 to $8.06 from 1981 to 1984, inclusive. The coefficient of the dummy variable (183) means that the deflated price rose 183 cents more than we would expect. In other words, there was an additional real price increase of 68.8 percent that occurred outside of historical demand.

The positive dummy coefficient leads us to conclude that there has been no substantial replacement of crab analog for king crab. Had a substantial replacement occurred, we would observe a negative dummy coefficient. It appears that the assumption of an increased demand for the kamaboko product due to high king crab prices may not be well founded. While this analysis adds credence to the supposition that crab analog is not a substitute or "imitation" for king crab, the results are not conclusive for other species of crab. Vondruska (1985) suggests that competition from other products, as measured by market shares, affects snow crab prices much more than king crab or blue crab, *Callinectes sapidus*, prices.

Discussion

The (0,1) dummy coefficient is certainly not the most efficient method to measure substitution effect. Had price data been available for crab analog imports, we could have measured direct price cross-flexibility where analog prices would appear as a parameter in our equation similar to snow crab prices. The inherent problem in using the dummy parameter is that it is most likely capturing more than our two assumed causal factors, a new marketing strategy, and competition from a new product. After all, there is an indeterminate number of factors which may affect market demand in any given period. Also, the estimation of a positive coefficient for the dummy variable does not preclude that substitution has occurred. We are merely concluding that the amount of substitution has not

[4]An asterisk indicates significance at the 0.10 level.

been substantial enough to outweigh the sum of other factors that have caused an additional increase in predicted demand for crab meat over the period.

Notwithstanding, the results of the equation do question conclusions reached by those people in the public and private sectors who believe that consumers en masse are substituting crab analog for crab meat. We feel that potential entrepreneurs in the United States should recognize that kamaboko may be prepared from fresh fish, and if the fish is available in large quantities continuously, it may be more profitable to forego the surimi process. There is a real need for further and continuing economic analyses of these dynamic product markets by government institutions and the private sector. With adequate preinvestment planning, there may be a bright future for a U.S.-based kamaboko industry.

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Products and Markets for Small Louisiana Shrimp

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ABSTRACT—Louisiana has long been known for its sometimes controversial harvest of small shrimp from its inshore waters. With the trend toward landings of smaller shrimp from the Gulf, the economics of the small shrimp processing and marketing industry is of increasing importance. The production of three shrimp products, raw head-on, peeled, and canned, was found to be dependent on small-shrimp supply. Over 20 percent of the shrimp moving through Louisiana's processing plants was shipped out of the state for further processing. Most of these were small, head-on shrimp. Louisiana is an important supplier of small-shrimp products and small shrimp for processors in other Gulf states.

Introduction

The Louisiana shrimp industry is undergoing a period of change because of increased competition and changes in shrimp markets. Shrimp imports have increased rapidly, rising 180 million pounds between 1976 and 1985. The percentage of the total U.S. shrimp supply accounted for by imports increased from 50 to 70 percent (U.S. Department of Commerce, 1986a) during that period. This increase in canned imports was even greater, increasing from 11 to over 80 percent (U.S. Department of Commerce, 1986a). These canned imports, mainly from Thailand, compete directly with Louisiana canned shrimp.

Historically, the shrimp industry in Louisiana has had its foundation in the harvest of small shrimp from the state's inshore waters. This is an important difference between Louisiana's and other Gulf states' shrimp industries. This has been a controversial practice, as some fishery managers and industry members argue that the shrimp should be allowed to grow to larger sizes. It has been proposed that this would improve the financial situation of the entire industry.

Yet in any reallocation of resources there are always those that gain and others that lose. A management plan that would increase the size of shrimp harvested from Louisiana's waters would have to decrease the shrimp available to the 13,000 inshore shrimpers and reallocate the landings to the 1,700 offshore shrimpers (Roberts and Pawlyk, 1986). The inshore fleet is composed of smaller state-registered boats that are not able to shrimp in deeper waters where the larger, offshore Coast Guard-documented vessels currently fish.

Studies have been done of the large and varied Louisiana shrimping fleet (Roberts and Sass, 1980; Roberts and Sass, 1979; Sass and Roberts, 1979), but none have examined the shore-based facilities of the state. When investigating management plans, it is important to have baseline information that this study provided. For example, the Gulf Council's shrimp management plan, in cooperation with the state of Texas, instituted a program designed to delay harvest of brown shrimp in order to increase shrimp size at harvest. The shrimpers and processing industry are forced to forego income during the closure in hope of larger income after the season opens. Analysis done by the National Marine Fisheries Service (NMFS) has shown that the value of landings is greater with the closure (personal communication, anonymous reviewer). It has not been documented whether this increase in value of landings compensates for all the costs caused by the closure and the delayed income.

Caillouet and Patella (1978) reported that there was evidence of an increase in the proportion of shrimp landed in the 68-and-over size category of shrimp in Texas and Louisiana. Another study reported that from 1950 to 1980, the Gulf supply of shrimp in this size category increased more than three times (Vondruska, 1984), while landings from the Gulf region increased only 40 percent (U.S. Department of the Interior, 1965; U.S. Department of Commerce, 1981). These increases in supply provide another reason why the products and markets for small shrimp should be better understood.

Another purpose for studying the uses of small shrimp was to determine their importance in creating employment in Louisiana. Shrimp has the highest total value of all Louisiana fisheries. The extensive shrimp industry is located in the state's southern parishes. With the decline of the oil-based economy in this area, interest is growing in promoting industries that are based on renewable resources to create employment. The effects of adding value to small shrimp through processing and the employment that this creates should be understood.

Methods

Data were collected from Louisiana shrimp processing and marketing firms using personal interviews. The 1984 survey covered 1983 calendar year operations. The firms were first stratified by location. They were located

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throughout southern Louisiana, from Cameron in the west to Venice in the Mississippi River delta. Some of the firms were located in metropolitan New Orleans, but the majority were found in much smaller towns. The surveyed firms were also stratified by the types of shrimp products that they were thought to produce so that an adequate sample of each type of product would be included in the survey. The 31 selected respondents accounted for 59 percent of the volume of Louisiana's shrimp landings as reported by the NMFS (U.S. Department of Commerce, 1986c) and 39 percent of the 80 land-based shrimp firms thought to be located in Louisiana.

For the purposes of this study, small shrimp were defined as those that were >50 count, headless, per pound. This definition was made after consultation with individuals knowledgable about the Louisiana shrimp industry, especially the canning sector. Of the approximately 42 million pounds of product sold by the 31 firms in the survey, 65 percent, or 27.4 million pounds, were from small shrimp, the size with which Louisiana differentiated itself from the other Gulf states.

Firm Characteristics

The firms in Louisiana's shrimp industry were divided into two distinct types: 1) Handlers, which produce only raw head-on or raw headless shrimp, and 2) processors, which process some or all of their shrimp into frozen-headless, peeled, canned, dried, and breaded products. On the basis of this classification, 39 percent of the surveyed firms were handlers and the remainder, processors (Table 1). The firms were also classified by size according to their dollar value of sales: Small firms had <$3 million in sales, medium firms had $3-5 million in sales, and large firms had sales >$5 million.

The firms also were divided into two other classifications: Those that utilized more than 50 percent small shrimp and those firms that utilized less than 50 percent. This resulted in 19 firms in the small-shrimp utilization category. Two thirds or more of the shrimp purchased by these firms were smaller than 50-count headless to the pound. This classification was used to examine the differences that might occur between firms dependent on small shrimp and the rest of the industry.

Table 2 illustrates some of the descriptive differences among the size classifications of the shrimp firms. Average sales were just under $5 million, with a wide range from small to large firms. In 1983, handlers averaged $2.8 million in sales and processors $6.2 million. Average age of the firms was 22 years, with little variation by firm size. However, processors averaged 29 years in business, while handlers averaged only 10 years. It was determined that many of the handlers began business by purchasing an already existing shrimp handling business. The turnover of these firms accounts for their shorter time in the business.

The investment information in Table 2 provides further insight into the relative newness of the handling firms. Many of these firms are small. While the average initial investment for all firms was over $450,000, initial investment for the small firms was only $150,000, making them easier to purchase. The initial investment for medium-sized firms was more than three times that of the small firms and that for large firms was 10 times that of the small firms. The ratio of owner's equity to borrowed capital gives the relative amounts that the investor must finance in order to get into the shrimp industry. Entry was easier with small firms. The prospective investor could provide more of the total capital necessary to begin business than the investor needed to finance from banks or other sources. The situation for large-sized firms was reversed. It was necessary to borrow almost all of the needed capital to start up a large shrimp firm. These factors account for the relative newness of the small shrimp handlers in Louisiana's shrimp industry.

Employment

A total of 1,167 people, full and part-time, was employed during the season by the 31 firms surveyed. These firms represent an important source of seasonal employment in the small coastal towns where they are located. It was estimated that 1,978 people were employed by the entire Louisiana shrimp processing and handling industry during the shrimping season, judging from the percentage of landings covered by the survey. Table 3 details the employment by position and by whether these were year-round or seasonal jobs.

There were very few year-round positions provided by these firms, with an overall average of eight per firm. There was little variation in this figure by size of firm. There was a wide variation in the number of seasonal employees by size of firm. Small firms hired just over

Table 1.—Number of firms surveyed by type and size of firm.

Firm type	All firms	Small	Medium	Large
Handlers	12	7	5	0
Processors	19	7	7	5
Total	31	14	12	5

Table 2.—Average characteristics of the Louisiana shrimp industry, 1983.

Item	All firms	Small	Medium	Large
Sales (million dollars)	$4.9	$2.6	$4.3	$14.1
Age of firm (years)	22.0	20.4	23.7	22.2
Investment	$469,000	$150,000	$560,000	$1,700,000
Equity/borrowed ratio	0.303	1.38	0.358	0.055

Table 3.—Average number of employees of Louisiana's shrimp industry by size of firm and type of position, 1983.

Position	All firms	Small	Medium	Large
Year-round				
Management	2.6	2.1	2.9	3.2
Plant Workers	4.5	5.2	3.6	4.6
Clerical	0.8	0.4	0.9	1.6
Sales	0.2	0.0	0.4	0.0
Subtotal	8.1	7.7	7.8	9.4
Seasonal				
Plant Workers	29.4	14.3	26.8	78.0
Clerical	0.2	0.1	0.0	0.8
Subtotal	29.6	14.4	26.8	78.8
Grand total	37.7	22.1	34.6	88.2

14 seasonal workers, while the large firms hired nearly 80 per plant. The overall average was just under 30 seasonal employees.

An important consideration for the state of Louisiana is the amount of employment created by firms dependent on small shrimp for their operations. It has been argued that firms that utilize small shrimp create more jobs than those that rely on larger shrimp. The firms were divided into two groups as described in the methods section of this paper; the 19 firms that used more than 50 percent small shrimp (>50 count headless) for their inputs were compared with the 12 that used less than 50 percent small shrimp. The small-shrimp-dependent firms had an average of 7.6 year-round positions and 29.7 seasonal jobs per plant. Those firms that were not small-shrimp-dependent had 8.7 year-round jobs per plant and 29.5 seasonal jobs. On the average, small-shrimp-dependent firms created no more employment than the other firms.

When examining employment, special attention should be paid to the firms with the greatest numbers of employees per firm, the canneries. All of the canneries fell into the small-shrimp-dependent category and were responsible for many of the jobs created by small-shrimp-dependent firms. Canneries averaged 14.6 year-round positions and 85.6 seasonal jobs. Firms other than canneries that were dependent on small shrimp had only 5.1 year-round and 14.9 seasonal positions per plant.

The closing of a cannery or other large shrimp processing plant can have varying effects on the local economy, depending on the firm's location. The loss of a plant in metropolitan New Orleans would not affect that area's economy as greatly as the closing of a firm in one of the smaller towns located throughout southern Louisiana. The loss of plants in such depressed areas would have much more serious consequences for the local economy.

Table 4 details the total employment created by the surveyed firms. Only 21 percent of the positions are year-round. The seasonal employment created by the shrimp industry can have an important impact on southern Louisiana. The data in Table 5 have been divided according to dependence on small shrimp. This illustrates the importance of small shrimp on southern Louisiana. The small-shrimp-dependent firms account for 61 percent of the employment in the surveyed firms. About 60 percent of the 709 jobs created by the surveyed small-shrimp-dependent firms were in the five firms that canned shrimp.

Shrimp Supply

The sources of supply for small shrimp used by Louisiana's shrimp firms are detailed in Table 6. Over 75 percent of the shrimp were unloaded at the firm's dock, with about 5 percent of total shrimp supply coming directly from company-owned vessels. The remaining shrimp were unloaded from independent shrimpers. Transhipments (shipments from other Louisiana shrimp firms) accounted for just over 15 percent of the small shrimp supply. About 5 percent of small-shrimp supplies were from brokers and other U.S. firms. Less than 0.5 percent of small shrimp was imported. This is in contrast to Florida, where only 35 percent of the shrimp purchased for processing was supplied by Florida's shrimpers (Prochaska and Andrews, 1974).

Sources of supply of small shrimp varied by size of firm. As the size of the firm increased, the reliance on its own dock for supply of small shrimp decreased. Some of the large firms did not operate dock facilities, but trucked in all shrimp needed for processing. The large firms relied more on transhipments and out-of-state sources for shrimp supplies. Surprisingly, even though large firms landed less shrimp at their docks, they did not use any imported small shrimp. Imports accounted for a very small percentage of the small shrimp used by Louisiana's shrimp industry. Florida's shrimp industry imported 40 percent of the volume of shrimp processed in that state (Alvarez et al., 1976).

The sources of supply of small shrimp did not vary significantly from the sources of all shrimp used by the Louisiana shrimp industry (Roberts and Pawlyk, 1986a). With the quantities of shrimp imported by the United States increasing every year since 1980 (452.2 million pounds in 1985; NMFS, 1986), shrimp of other sizes are readily available to the Louisiana shrimp industry. Shrimp are also available from other states. These sources were not found to be important to Louisiana's shrimp industry. The industry remains dependent on small shrimp.

Products and Marketing Channels

The shrimp products sold by the surveyed firms were as follows: Fresh head-on, fresh headless, frozen headless, canned, peeled, breaded, and

Table 4.—Total employment in surveyed firms, by size of firm and type of position.

Firm size	Year-round	Seasonal	Total
Small	108	202	310
Medium	94	322	416
Large	47	394	441
Total	249	918	1,167

Table 5.—Total employment in surveyed firms, by dependence on small shrimp.

Firm type	Year-round	Seasonal	Total
Not dependent on small shrimp	104	354	458
Small-shrimp dependent			
Canneries	73	355	428
Other	72	209	281
Subtotal	145	564	709
Grand total	249	918	1,167

Table 6.—Sources of supply of small shrimp for Louisiana's shrimp industry by size of firm, 1983.

Source	All firms	Small	Medium	Large
Company vessels	5.3	10.7	0.3	3.0
Independent vessels	72.6	84.2	66.9	53.4
Subtotal	77.9	94.9	67.2	56.4
Transhipments	16.5	2.7	30.9	20.0
Brokers	3.2	0.7	0.0	18.0
Other U.S. firms	1.7	0.1	1.9	5.6
Imports	0.4	0.9	0.0	0.0
Grand total[1]	100.0	100.0	100.0	100.0

[1]May not add to 100 percent due to rounding.

Figure 1.—Market areas for Louisiana shrimp.

Table 7.—Percent and volume (in million pounds) of use of small shrimp (>50 count, headless) by product type by surveyed Louisiana firms, 1983.

		Small shrimp	
Product form	Total volume	Percent	Volume
Head on	11.2	73.6	8.2
Headless	13.2	28.3	3.9
Canned	9.1	100.0	9.1
"Other"	8.2	77.5	6.2
	42.1		27.4

Table 8.—Market channels for Louisiana shrimp products in percent of total pounds sold, 1983.

	Product form			
Market area	Head on	Headless	Canned	Other
Louisiana	8.7	6.0	5.1	4.6
Other Gulf	16.5 (15.4)[1]	5.0 (2.7)	2.0	3.1
Southeast	0.5 (0.5)	2.5 (0.6)	2.7	2.7
Northeast	0.9 (0.0)	9.5 (1.4)	2.5	6.6
Midwest	0.0 (0.0)	7.2 (1.6)	2.8	0.9
West	0.1 (0.1)	1.5 (0.1)	3.6	0.8
Export	0.0 (0.0)	1.3 (0.2)	2.9	0.1
Total	26.7 (16.0)	32.9 (6.6)	21.6	18.9

[1]Percent of total production further processed in each market area. For descriptions of market areas, see Figure 1.

dried. Fresh and frozen headless shrimp were combined into one product type, headless shrimp, because of their similarities and the relatively small amount of fresh headless shrimp produced (5.5 percent of total production). Peeled, breaded, and dried shrimp were combined into the category of "other" shrimp, since the latter two products represented only 2 percent of total volume sold by the surveyed firms and were sold by one and two of the firms, respectively.

The relative importance of small shrimp by product type is depicted in Table 7. These calculations were based on the amount of >50 count, headless shrimp used in each product category. Three products, head-on, canned, and "other," were over 70 percent small shrimp. Peeled shrimp were 77 percent small.

Since there has been increasing interest in adding value to seafood products through processing to create employment in Louisiana, it is interesting that the least processed product, head-on shrimp, and the two most processed products, canned and "other" shrimp, are all dependent on small shrimp.

The market channels for Louisiana's small shrimp were investigated by asking the surveyed firms to provide information on the locations of their sales by type of product. Other Gulf states become involved in the processing of small shrimp through the receipt of small Louisiana raw shrimp. To identify the amount of shrimp that leaves Louisiana to be processed, the other Gulf states and Louisiana were designated as separate market areas. This was done because it was hypothesized that raw shrimp shipped out of Lousiana would ultimately be processed in other Gulf states. The remaining markets in the shrimp product distribution system were identified as large regions (Fig. 1). Alaska and Hawaii were considered part of the western market. Any shrimp shipped out of the United States was designated as exported.

The Louisiana shrimp production that was marketed in each area by product category is identified in Table 8. Table 8 also contains the percent of total production that is further processed outside Louisiana. Almost 27 percent of the 42 million pounds sold by the surveyed firms was sold as raw, head-on shrimp. Over 60 percent of the total head-on volume went to the other Gulf states. On a headless basis, this amounted to 6.9 million pounds of raw, head-on shrimp in 1983, of which 5.1 million pounds were small. Survey data were used to estimate corresponding figures for all Louisiana firms based on 1983 landings. Even though the surveyed firms represented 38 percent of the shrimp firms located in the state, it was thought that since they accounted for 59 percent of the landings, that the latter figure would be more appropriate for estimating the total volumes for each product sold. Of the fresh Louisiana head-on shrimp sold, 11.8 million pounds were shipped to the other Gulf states with 10.9 million pounds being processed there. About 8.1 million of the 10.9 million pounds was classified as small. A total of 11.4 million pounds of head-on shrimp were further processed outside of Louisiana, with 8.4 million pounds classified as small.

Headless shrimp had the lowest percentage and volume of small shrimp of the four product classifications. At the time of the survey, there was not a favorable market for small headless shrimp. There was less out-of-state processing done on headless shrimp than was done to the head-on shrimp. A total

Table 9.—Number of Gulf canned shrimp processing plants, total Gulf canned production, and imports, 1960-85 (NMFS data).

Year	Number of plants	Canned shrimp (millions of cases)	
		Domestic	Imported
1960	34	1.9	NA[1]
1961	33	1.1	NA
1962	34	1.7	NA
1963	31	2.2	0.6
1964	26	1.3	0.4
1965	28	2.2	0.3
1966	28	1.9	0.2
1967	29	2.0	0.3
1968	28	2.0	0.6
1969	26	2.0	0.5
1970	24	2.5	0.6
1971	23	2.1	0.4
1972	21	2.2	0.2
1973	21	2.0	0.4
1974	20	1.9	0.9
1975	19	1.0	0.2
1976	16	1.7	0.4
1977	15	2.1	0.4
1978	14	1.5	0.6
1979	13	0.9	0.6
1980	13	1.8	0.6
1981	12	1.1	0.7
1982	11	0.7	0.8
1983	12	1.0	2.0
1984	11	0.9	2.0
1985	10	0.5	2.5[2]

[1]Not available.
[2]Preliminary.

of 9.4 million pounds of headless shrimp were shipped out of Louisiana for further processing by all Louisiana firms. Of this, 1.5 million pounds were small shrimp.

Louisiana supplied 9.9 million pounds of small shrimp to other states for further processing, which was 5 percent of the 1983 Gulf landings of shrimp. Louisiana is an important supplier of small shrimp used in processing plants around the Gulf. However, employment that might be created by the processing of these shrimp is being lost by Louisiana.

While head-on shrimp were distributed to only two of the market areas, canned and "other" shrimp were sold by the surveyed firms to all of the market areas including the export market. Canned shrimp were evenly distributed throughout the market areas. A larger proportion of "other" shrimp was shipped to the northeast, less to other areas. Based on estimates for all Louisiana firms, canned shrimp accounted for 15.4 million pounds of the small processed shrimp in 1983. Total pounds of "other" shrimp sold were 13.6 million headless, with 10.4 million pounds classified as small. The exports of canned Louisiana shrimp accounted for 27 percent of the 7.6 million pounds of canned shrimp exports in 1983 (U.S. Department of Commerce, 1986a). Even though the volume of Gulf shrimp landings increased from 126 million pounds in 1983 to 162 million pounds in 1984 (U.S. Department of Commerce, 1986b), the amount of shrimp canned in the Gulf fell from 937,000 cases to 819,000, and fell further in 1985 to 548,000 (U.S. Department of Commerce, 1986c), even though Louisiana's shrimp harvest set a new record (74 million pounds, U.S. Department of Commerce, 1986c). The decline in canning must have been caused by factors other than quantity of landings in the needed sizes. This is another indication of the decline in the U.S. shrimp canning industry.

One factor contributing to the decrease in Gulf canned pack shrimp is increasing competition from imported canned shrimp (Table 9), especially from Thailand. The first year that canned shrimp imports exceeded Gulf canned pack was 1982. An expanding seafood canning industry in Thailand has been cited as an important factor in the increased imports of canned shrimp in the United States market.

Firm Concentration

Another way to examine Louisiana's small shrimp industry is to analyze the concentration of production using the Lorenz curve analysis. A Lorenz curve is a continuous function that graphically depicts the relationship between the percentage of firms and the percentage of sales made by these firms. If each firm has an equal share of sales, the Lorenz curve will appear as a diagonal line originating from the origin of the graph. An industry with most of the production coming from one large firm will appear as a concave line, with the line "bowed" toward the right hand side of the graph. The more concentrated the industry, the further the Lorenz curve will "bowed" to the right, away from the diagonal line.

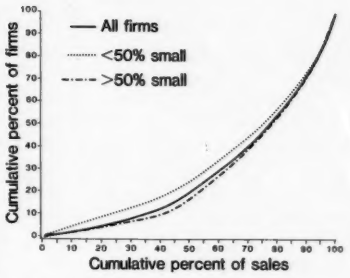

Figure 2.—Lorenz curves for the Louisiana shrimp industry by percent of small shrimp used, 1983.

It was hypothesized that the production from firms that utilized mostly small shrimp would be concentrated in fewer firms than those that processed larger shrimp, because of the presence of large canners in the small-shrimp-dependent group. To examine this hypothesis, the surveyed firms were divided into two groups according to their dependence on small shrimp, as described in the Methods section. The shrimp purchased by 12 of the firms consisted of <50 percent small shrimp, while the remaining 19 firms' shrimp purchases were >50 percent small. As can be seen from Figure 2, the hypothesis was supported. Those firms that used more than 50 percent small shrimp were more concentrated than those that were less dependent on these sizes.

A factor contributing to the concentration of the firms dependent on small shrimp is the increasing concentration of canners in the Gulf. As can be seen in Table 9, the number of shrimp canners in the Gulf has been steadily decreasing over time. There were only one-third as many canners in 1984 as there were in 1960.

Perceived Problems

The Louisiana small shrimp processing and marketing industry is not without its problems. The shrimp industry has been faced with increasing competition from imports. When asked to identify factors that would affect firm growth in the future, imported and cultured shrimp accounted for 29 and 16

percent of the answers, respectively. This is not surprising given the rapid increase of shrimp imports in recent years and the industries' dependence on domestic supplies of shrimp. Canneries were especially concerned with the rapidly rising imports of inexpensive canned shrimp, especially from Thailand. One difficulty reported by some of the canners is that the smallest size category of canned shrimp does not have a minimum size. Thus, Thai canned shrimp can be smaller than domestic canned shrimp and still stay within Food and Drug Administration regulations. This, coupled with lower labor costs, gives the canned imports a price advantage. Overall supply of shrimp and supply of shrimp in certain sizes was also a major concern of Louisiana's shrimp industry. These categories accounted for 22 and 9 percent of the responses, respectively. It is important to note that none of these problems can be directly controlled by the individual firms or the industry itself.

Discussion

Small shrimp are important to the economy of southern Louisiana. About 27.3 million pounds or 65 percent of all shrimp sold in 1983 by the 31 firms surveyed were small (<50 count headless). Out of this amount, 15.3 million pounds were processed into canned shrimp (9.1 million pounds) or "other" shrimp (6.2 million pounds). Canneries accounted for many of the almost 1,200 full and part-time jobs created by the surveyed firms. With a steady decline in both Gulf canneries and canned shrimp, and with rising imports in this product category, canners are declining as a source of employment for southern Louisiana. The only other Louisiana products in which small shrimp were used in large amounts were "other" shrimp (mostly peeled), and raw head-on. Much of the raw head-on shrimp was shipped from the state to be processed elsewhere. If the percentage of shrimp leaving Louisiana for processing increases from its current level of 22.6 percent, additional jobs will be lost in Louisiana.

The survey of firms did not gather any data on whether the peeled market can be expanded to compensate for the decline of the domestic canned market. Two indications that it may expand are: 1) All but one of the canneries also sold peeled shrimp; thus they have some experience with this product and may be able to redirect their marketing efforts to peeled shrimp products. And 2) at least three new shrimp peeling firms began operation in Louisiana in 1986.

Only one of the problems that the industry identified, the supply of shrimp in certain sizes, can be affected by the state's management of the shrimp resource. There would be winners and losers in any change in the current management scheme. If management moved toward larger shrimp, Louisiana firms that sell headless shrimp will have a greater supply of shrimp. The supply of shrimp for peeling would decline, affecting this market, which is attracting new firms. A decline in quantities of small shrimp would also adversely affect the canners, whose market is already in decline. Total employment would decline since canneries hire a large percentage of the seasonal workers employed by the industry. This could have a severe affect on the economy of the small, southern Louisiana towns where many of these firms are located. Other changes in total employment would be caused by changes in the number of firms operating in Louisiana. The shrimp resource would reallocate from the 13,000 shrimp trawlers and the 4,000 butterfly netters that operated in the state's inshore waters in 1985 to the 1,700 larger vessels that shrimped offshore (Roberts and Pawlyk, 1986), which would again lessen employment.

The Louisiana small-shrimp-dependent industry must adapt to the changing conditions caused by increased competition and changes in the markets for shrimp. It is hampered by the fact that none of these changes are directly under the control of the firms or the industry. How the industry and managers react to these changes will have lasting effects on the economy of southern Louisiana.

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NMFS Outstanding Publications Cited

Winners of the National Marine Fisheries Service's Outstanding Publications Award for papers published in the *Marine Fisheries Review* (vol. 46) and the *Fishery Bulletin* (vol. 82 and 83) have been announced by NMFS Publications Advisory Committee Chairman Ben Drucker, along with special recognition of a nine-paper section in the *Marine Fisheries Review* entitled "The Status of Endangered Whales."

"Groundfish Fisheries and Research in the Vicinity of Seamounts in the North Pacific Ocean" by R. N. Uchida and D. T. Tagami of the NMFS Southwest Fisheries Center's Honolulu Laboratory was selected by the Awards Committee as the best paper in the *Marine Fisheries Review*, 46(2):1-17. Selected as the best papers in the *Fishery Bulletin* for volumes 82 and 83, respectively, were: "Morphology, Systematics, and Biology of the Spanish Mackerels (*Scomberomorus*, Scombridae)" by Bruce B. Collette and J. L. Russo, 82(4):545-692; and "Using Objective Criteria and Multiple Regression Models for Age Determination of Fishes" by George W. Boehlert, 83(2): 103-117. Collette is with the NMFS National Systematics Laboratory in Washington, D.C., and Boehlert is with the NMFS Southwest Fisheries Center's Honolulu Laboratory.

In all, six papers were nominated from volume 82 of the *Fishery Bulletin*, seven papers from volume 83, and six papers were nominated from volume 46 of the *Marine Fisheries Review*, along with the nine whale papers.

Earning special recognition from the Awards Committee was a collection of nine papers in the *Marine Fisheries Review*, 46(4):1-64, entitled "The Status of Endangered Whales" which was prepared by scientists with the National Marine Mammal Laboratory at the Northwest and Alaska Fisheries Center, Seattle, Wash. Papers in that collection, which were edited by Jeffrey M. Breiwick and Howard W. Braham, included: "The Status of Endangered Whales: An Overview," by Howard W. Braham; "The Gray Whale, *Eschrichtius robustus*," by Dale W. Rice, Allen A. Wolman, and Howard W. Braham; "The Blue Whale, *Balaenoptera musculus*," by Sally A. Mizroch, Dale W. Rice, and Jeffrey M. Breiwick; "The Fin Whale, *Balaenoptera physalus*," by Mizroch, Rice, and Breiwick; "The Sei Whale, *Balaenoptera borealis*," also by Mizroch, Rice, and Breiwick; "The Humpback Whale, *Megaptera novaeangliae*," by J. A. Johnson and Allen A. Wolman; "The Right Whale, *Balaena glacialis*," by Braham and Rice; "The Bowhead Whale, *Balaena mysticetus*" by Braham; and "The Sperm Whale, *Physeter macrocephalus*," by Merrill E. Gosno, Rice, and Breiwick.

The five other papers nominated in volume 82 of the *Fishery Bulletin* were: "Spring and Summer Prey of California Sea Lions, *Zalophus californicus*, at San Miguel Island, California" by G. A. Antonelis, C. H. Fiscus, and R. L. DeLong, 48(1):67-76; "Age, Growth, and Mortality of Gray Triggerfish, *Balistes capricus*, From the Northeastern Gulf of Mexico" by A. G. Johnson and C. H. Salomon, 82(3):485-492; "Calibration of Dental Layers in Seven Captive Hawaiian Spinner Dolphins, *Stenella longirostris*, Based on Tetracycline Labeling" by A. C. Myrick, Jr., E. W. Shallenberger, I. Kang, and D. B. MacKay, 82(1):207-225; "Documentation of Annual Growth Lines in Ocean Quahogs, *Arctica islandica* Linne" by J. W. Ropes, D. S. Jones, S. A. Murawski, F. M. Serchuk, and A. Jearld, Jr., 82(1):1-19; and "Selection of Vegetated Habitat by Brown Shrimp, *Penaeus aztecus*, in a Galveston Bay Salt Marsh" by R. J. Zimmerman, T. J. Minello, and G. Zamora, Jr., 82(2):325-336.

The six other papers nominated from volume 83 were: "Dolphin Habitats in the Eastern Tropical Pacific" by D. W. K. Au and W. L. Perryman, 83(4):623-643; "Confidence Limits for Population Projections When Vital Rates Vary Randomly" by T. Gerrodette, D. Goodman, and J. Barlow, 83(3):207-217; "Egg Production of the Central Stock of Northern Anchovy, *Engraulis mordax*, 1951-82" by N. C. H. Lo, 83(2):137-150; "Long-term Responses of the Demersal Fish Assemblages of Georges Bank" by W. J. Overholtz and A. V. Tyler, 83(4):507-520; "The Rock Shrimp Genus *Sicyonia* (Crustacea: Decapoda: Penaeoidea) in the Eastern Pacific" by I. Perez Farfante, 83(1):1-79; and "Age, Growth, and Distribution of Larval Spot, *Leiostomus xanthurus*, off North Carolina" by S. M. Warlen and A. J. Chester, 83(4):587-599.

The five other MFR papers nominated from volume 46 were: "Using Charterboat Catch Records for Fisheries Management" by H. A. Brusher, M. L. Williams, L. Trent, and B. J. Palko, 46(3):48-55; "Dungeness Crab Leg Loss in the Columbia River Estuary" by J. T. Durkin, K. D. Buchanan, and T. H. Blahm, 46(1):22-24; "U.S. Tuna Trade Summary, 1983" by S. F. Herrick, Jr., and S. Koplin, 46(4):65-72; "Assessing the Accuracy of a Method to Determine the Amount of Minced Fish in Mixed Mince-Fillet Fish Blocks" by J. P. Lane, J. J. Ryan, and R. J. Learson, 46(3):76-79; and "Fish or Fish Oil in the Diet and Heart Attacks" by M. E. Stansby, 46(2):60-63.

Developed in 1975, the annual outstanding publication awards program recognizes NMFS employees who have made exceptional contributions to the knowledge and understanding of the resources, processes, and organisms studied as a part of the NMFS mission. Authors must have been employed by the NMFS at the time the paper was published. *Marine Fisheries Review* papers must be effective and interpretative contributions to the understanding and knowledge of NMFS mission-related studies, while *Fishery Bulletin* papers must document outstanding scientific work.

At the close of each volume, nominations are solicited from the NMFS Center, Regional, and Office Directors for the the awards by the Awards Com-

mittee Chairman—the editor of the *Fishery Bulletin*, currently Andrew Dizon at the NMFS Southwest Fisheries Center. Other Committee members include the editor of the *Marine Fisheries Review*, W. L. Hobart, and former *Fishery Bulletin* editors Bruce B. Collette, Reuben Lasker, Jay Quast, William J. Richards, and Carl Sindermann.

Data Resources of the NODC

The National Oceanographic Data Center (NDC), which this year celebrated its 25th anniversary, is the U.S. national repository and dissemination facility for global oceanographic data. Established in 1961 as an interagency facility uner the management of the Naval Hydrographic (now Oceanographic) Office, the NODC has been part of NOAA since NOAA was created in 1970. Today the NODC operates within the NOAA National Environmental Satellite, Data, and Information Service (NESDIS). The environmental data files of the NODC and its sister centers (the National Geophysical Data Center (NGDC), Boulder, Colo., and the National Climatic Data Center (NCDC), Asheville, N.C.) are installed on a central NESDIS computer facility located at the NCDC in Asheville.

Through the NODC, researchers have access to data from government agencies, universities and research institutions, industry, and foreign agencies and organizations. Foreign data are acquired through bilateral and multilateral exchanges. Since 1962 the U.S. NODC has operated World Data Center A for Oceanography, one of the U.S. components of a global network that facilitates international data exchange. The NODC receives data from dozens of other countries including the U.S.S.R. and other eastern-bloc nations and the People's Republic of China.

NODC's primary resources are its archive data files. Data received by NODC that are processable into standard formats enter its data processing/quality control system and are merged into the appropriate data files. Data in these files can be selectively retrieved (either by cruise or by geographic area and time period) and provided to customers in a variety of media and forms. Besides printouts and magnetic tape copies of selected data, the NODC provides a wide selection of customized data summaries, analyses, and graphic plots. All NODC data products are provided at cost. Standard charges are imposed for some relatively simple products; cost estimates are provided for more complex jobs.

For certain standard types of ocean data such as hydrographic stations and bathythermograph temperature profiles, NODC data files have worldwide coverage. NODC holds other types of physical, chemical, and biological data primarily for U.S. offshore and outer continental shelf areas. For example, the NODC receives and archives wind and wave data from automated buoys operated by the NOAA National Data Buoy Center. NODC's physical/chemical data files (Table 1) include ocean temperature and circulation data that are finding increased application to climate studies. A computerized data inventory system enables NODC personnel to respond quickly to customer inquiries about the quantity of available data meeting specified selection criteria.

In addition to its archive files, NODC also provides copies of special data sets held in originator formats. For example, NODC holds data tapes prepared by S. Levitus, NOAA/GFDL, in conjunction with the "Climatological Atlas of the World Ocean." These tapes include global objective analyses of major ocean parameters on a one-degree grid.

From the NOAA Ocean Products Center (OPC) the NODC is receiving quality-controlled marine data products. Global Blended Sea Surface Temperature analyses (which combine in situ and satellite data prepared in support of the Tropical Ocean Global Atmosphere (TOGA) program) are now available at NODC. The initial data were received in December 1985. NODC is also receiving and archiving monthly IGOSS data tapes. These data are received at NODC within several working days after the end of each month. Detailed information about NODC's data holdings, products, and services is provided in the NODC Users Guide, which is

Table 1.—Major NODC physical/chemical data files.

Data type	Volume (as of 4/86
Oceanographic Stations	713,268 stations
Mechanical BT data	980,222 stations
Expendable BT data	531,799 statons
CTD/STD data (high resolution)	38,176 stations
CTD/STD data (low resolution)	36,368 stations
Surface current (ship drift) data	4,175,000 stations
Current meter data	17,292 obs.-mo.[1]
Lagrangian current measurments	1,258 obs.-mo.[1]
Coastal wave data	51 obs.-mo.[1]
NDBC buoy data	6,043 obs.-mo.[1]
Pressure gauge data	473 obs.-mo.[1]
Water physics and chemistry	71,538 stations
Marine toxic substances and pollutants	11,159 stations

[1]Time series data are reported as observation-months, i.e., measured parameters recorded for 1 month.

NODC and WDC-A Offices Move

The Washington, D.C., offices of the U.S. National Oceanographic Data Center (NODC) and the World Data Center A (WDC-A) for Oceanography have been moved. The new NODS/WDC-A address is: National Oceanographic Data Center, NOAA/NESDIS, 1825 Connecticut Avenue, N.W., Washington, DC 20235.

The new telephone numbers, which are both commercial and FTS, are as follows: Gregory W. Withee, Director, 202-673-5594; User Services Branch, 202-673-5549; WDC-A, Oceanography, 202-673-5571; Ocean Pollution Data and Information Network (OPDIN), 202-673-5539; and the Data Acquisition and Management Branch, 202-673-5643.

available free. Customers orders and inquiries should be directed to: National Oceanographic Data Center, User Services Branch NOAA/NESDIS E/OC21, Washington, DC 20235; Telephone, 202-634-7500 or FTS 634-7500; Telemail: Mailbox "NODC.WDCA". (Source: Richard J. Abram.)

Omega-3 Fatty Acids for Cardiovascular Research

Since the 1950's, scientists have recognized the unique properties of fish oils in preventing heart disease. Greenland Eskimos and Japanese fishermen are unusually resistant to atherosclerosis and, during World War II, Norwegians recovered from heart attacks more rapidly than previously. Both of these effects are attributed to consumption of fish, but the specific beneficial compounds are yet to be identified. The complex nature of fish oils has hampered isolation and evaluation of individual compounds for physiological activity.

Now, the Fish Oil Research Group of the NMFS Northwest and Alaska Fisheries Center's Utilization Research Division (URD) has found a way to prepare EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in purities up to 94 percent. EPA and DHA are the omega-3 fatty acids present in fish oils in the largest amounts. Omega-3 fatty acids, thought to inhibit the plaque formation and blood clotting responsible for heart attacks, are found in substantial quantities only in marine oils. Oils from terrestrial sources contain mainly omega-6 compounds which appear to contribute to cardiovascular disease. Consequently, availability of EPA and DHA in adequate quantities will facilitate the biochemical and clinical studies needed to elucidate the exact physiological properties that make fish oils beneficial to human health.

A state-of-the-art method, supercritical carbon dioxide fractionation, allows EPA and DHA to be separated from numerous other fatty acids normally present in fish oils. To expedite the process, short-chain saturated and monounsaturated fatty acids are removed selectively beforehand by urea complexation. In this way, the amount of material involved in the supercritical fractionation is reduced and also the effectiveness of the separation is enhanced. Supercritical extraction is already used to extract residual petroleum from oil wells and in the food industry to decaffeinate coffee. The advantage of supercritical carbon dioxide are low temperatures required, exclusion of oxygen, ready availability, low cost, nontoxicity, and absence of solvent residues.

To date, menhaden oil, the fish oil produced in largest quantities in the United States, has been used for our studies. It represents a good source of both EPA and DHA (Table 1). Because of the similarity of these two compounds, the presence of one makes isolation of the other more difficult. Now, however, we are investigating two regional resources which appear to have special potential as starting materials for the isolation of EPA and DHA. Alaska pollock liver oil is especially attractive for production of EPA, as are tuna oils for DHA production. Nonspawning pollock liver oil contains 17 percent EPA and 5 percent DHA. The high ratio of EPA to DHA, 3.5, should simplify isolation of EPA in high purity. Tuna oils are very attractive sources of DHA because of unusually high concentrations in the starting oils, 25-29 percent, and even more because of the low concentrations of EPA, 6.7-7.7 percent.

Pollock livers represent a vast untapped resource, a byproduct currently without a market. For maximum utilization, they should be collected from nonspawning animals because during spawning, the EPA content drops and the DHA content rises (Table 1). Tuna oils are recovered from the precook liquor and have little commercial value. Purification and production of these oils would dramatically enhance the value of the waste products. Production of EPA and DHA for biochemical and chemical studies and potentially for sale as nutritional supplements and pharmaceutical agents would provide attractive markets for two unused regional products. Source: Virginia F. Stout, URD, NWAFC, Seattle, Wash.

Table 1.—Fatty acid profiles of menhaden, Alaska pollock, and tuna oils.

Oil source	Palmitic 16:0	Oleic 18:1	EPA 20:5	DHA 22:6	EPA/DHA
Menhaden	16.8	9.4	16.0	8.4	1.9
Pollock liver					
Nonspawning	18.6	18.7	17.3	4.9	3.5
Spawning	15.9	18.7	15.6	6.4	2.4
Spawning	14.5	17.0	13.8	7.6	1.8
Tunas					
Albacore	17.7	13.7	7.7	29.4	
Skipjack	18.5	12.4	7.4	27.9	0.27
Yellowfin	17.7	15.1	6.7	25.3	0.26

Pressurized Containers for Dispensing Fish Oils

Pressurized containers show promise for easy, effective storage of fish products, reports the Utilization Research Division (URD) of the NMFS Northwest and Alaska Fisheries Center, which has been looking for a convenient means of storing and dispensing fish oils for clinical studies on their efficacy in preventing and treating cardiovascular disease. The physiologically active components of fish oils, omega-3 polyunsaturated fatty acids, decompose readily in air. That is one of the reasons for cod liver oil's taste, but flavor is not the only issue. Oxidation of the active components of fish oil reduces the potency; even more important is actual toxicity. Adding to the problem is the fact that the whole process, formally called autoxidation, is a chain reaction; once initiated, autoxidation keeps expanding as it goes. Oxygen in the air is the driving force.

Exclusion of oxygen is critical to the preservation of fish oil and omega-3 products. Until recently, two alternatives have been used for storage of fish oils: Sealed, evacuated glass ampoules and nitrogen-flushed, screw-cap bottles. Ampoules are tricky to open and only useful once, since they cannot be resealed easily. Bottles are more convenient for multiple use, but once opened, air is admitted. To avoid autoxidation, the remaining omega-3 material must be flushed again with purified nitrogen, a cumbersome procedure.

Pressurized packaging, so popular for cosmetics, paints and insecticides,

seems to be the method of choice for storing and dispensing fish oils. Preliminary experiments show that the oils are stable in pressurized cans. With ultrapure nitrogen as the propellant, metered doses are easily dispensed. Next, the applicability of the technique to omega-3 concentrates will be evaluated. Source: Virginia F. Stout, URD, NWAFC, Seattle, Wash.

U.S. Salmon Faces More Competition in Europe

European salmon farming was expected to reach record levels in 1986, with Norway, the leading supplier, expected to produce 40,000 metric tons (t) of farmed salmon in 1986. The other major European suppliers, Scotland and Ireland, should have 1986 production levels of 8,000 and 1,500 t, respectively. This level of production, coupled with weakening of the U.S. market for imported salmon, has resulted in lower prices in the German market for salmon. Fresh salmon competes with U.S. exports of frozen salmon.

In 1985, the United States was Germany's largest supplier of frozen salmon with 1,014.9 t. Other suppliers included Canada (790 t), Norway (174.7 t), and Scotland (9.6 t). Summertime German wholesale prices for Norwegian salmon were $3.13/pound compared with $3.55/pound for comparable quality frozen American salmon (at US$1 = DM2.18).

Although the fresh salmon enjoys both a competitive and price advantage over U.S. frozen salmon, German salmon importers stated that they would nevertheless be watching the U.S. salmon prices during the upcoming season. U.S. salmon exporters wishing to enter or maintain their market shares, should be aware of the current market situation in order to set their prices accordingly.

The Federal Association of the German Fish Industry and Fish Wholesale Trade has reported that Norwegian salmon farming is highly subsidized, although there is no information available on Norwegian salmon farming subsidies nor a calculation of the indirect effect of the subsidization on salmon exports to third country markets. The German fish industry association also speculates that the level of the subsidy might be reduced in the next 3-5 years, thereby bringing a corresponding increase in prices for Norwegian farmed salmon. [However, Norwegian authorities have stated in response that their salmon farming is not subsidized.] Source: USDC, Business America.

Cooperative Efforts to Save Endangered Species

A mid-July flight to Hawaii's French Frigate Shoals was made to assist in recovery of the "threatened" green turtle and the "endangered" Hawaiian monk seal, reports Richard S. Shomura, Director of the NMFS Southwest Fisheries Center's Honolulu Laboratory. A private conservation organization paid for the flight which took green turtle hatchlings to French Frigate Shoals for release to the wild and returned to Honolulu with a weaned but underdeveloped female monk seal pup for rehabilitation.

Shomura said the 46 turtle hatchlings were from Sea Life Park's captive turtle breeding program and were released at Tern Island at French Frigate Shoals where they crawled down the beach to enter the ocean. Hopefully, the turtles would imprint there and return someday to nest on that protected island. Tiny metal tags were placed on their flippers so that identification of them would be possible if they are resighted. Other Sea Life Park hatchlings were released on Oahu beaches earlier in the year.

Recovery of the small female monk seal is part of a project being conducted by the NMFS Marine Mammals and Endangered Species Program. According to William G. Gilmartin, Program leader, female pups like that are brought to Honolulu for "fattening" and then reintroduction into the wild at Kure Atoll in the northwestern Hawaiian Islands where the monk seal population is very depleted and in critical need of females. Three other females were collected earlier this year, flown to Honolulu, and were being cared for by NMFS. Gilmartin said five young females collected and rehabilitated in 1984 and 1985 had already been added to the Kure population.

Shomura also reported that a young green sea turtle which was found injured near Kailua-Kona during the summer was taken to Honolulu and treated, thanks to the efforts of several concerned citizens. The turtle was found by personnel aboard a private dive boat who had noticed that the animal was unable to submerge and had a hole in its shell just behind the head.

The divers turned over the turtle to Russell Yim, an agent with the Hawaii State Division of Conservation and Resource Enforcement. All sea turtles in Hawaii are protected, and illegal taking or possession can result in imprisonment and fines of up to $10,000. The turtle was held temporarily in a hotel pond until it could be treated.

"When we were notified," said John Henderson, NMFS Fishery Biologist, "we decided to fly the turtle to Honolulu for treatment. According to Patrick Leadbeater, Honolulu veterinarian, to whom the turtle was taken "the hole appeared to have been made by a spear or arrow." Said Leadbeater, "air had gotten into the animal's body cavity, making it too buoyant to submerge and feed. We treated the turtle with antibiotics and removed some damaged shell and muscle tissue from the wound. After we were confident that there was no infection, we patched the hole with a biologically compatible foam plug and a coating of fiberglass resin. If the air inside the cavity was coming from the hole, the turtle could biologically rid itself of the gas in a couple of weeks. But, if the lungs are damaged and leaking air into the body cavity, it would take a much longer time to heal."

"We have recovered many turtles during the last few years with spear holes, and most of them died," said William Gilmartin, Leader of the NMFS Marine Mammals and Endangered Species Program. Gilmartin believed the effort by many concerned people to get this turtle treated quickly could pay off and allow the turtle to survive. "Even though these turtles are protected by the Federal Endangered Species Act and State law as well," Gilmartin added, "the illegal taking of turtles makes recovery of the population extremely difficult." Gilmartin said the public can be a big help with this by reporting inci-

dents in which turtles are injured or collected by poachers to enforcement officers at NMFS (546-5670) or the State (548-5918, or outer islands, ask operator for toll free Enterprise 5469). The turtle was being kept at the NMFS Kewalo Research Facility, and, should it completely recover, it will be tagged and released, possibly back in the area where it was found.

Gulf Ichthyoplankton Samples for Research

The Gulf States Marine Fisheries Commission has announced the availability of Gulf of Mexico ichthyoplankton samples for loan to qualified researchers. Samples have been and are continuing to be collected for SEAMAP (Southeast Area Monitoring and Assessment Program), a multi-year international Federal/state/university program of the GSMFC. Neuston and bongo nets were employed for specimen collection in a one degree latitude/longitude grid over the entire Gulf from lat. 26°N northward; samples were sorted and preliminarily identified by the Plankton Sorting and Identification Center, Szczecin, Poland.

At present, samples from 1982 (7,057 lots, 93 families), 1983 (8,351 lots, 106 families), and material from one summer cruise in 1984 (4,155 lots, 75 families) are available for loan. Lots of unsorted fish eggs are also available from these years. Most samples are sorted to the family level, although many have identification to generic or species level. Additional 1984 samples are expected to become available by the end of 1986. Specimens are available for loan on a 6-month renewable basis. Researchers interested in obtaining additional information can contact either the SEAMAP Ichthyoplankton Curator, Florida Department of Natural Resources, Bureau of Marine Research, St. Petersburg, FL 33701, or the SEAMAP Coordinator, Gulf States Marine Fisheries Commission, P.O. Box 726, Ocean Springs, MS 39564.

This statement is required by The Act of August 12, 1970, Section 3685, Title 39, U.S. Code, showing ownership, management, and circulation of the Marine Fisheries Review, publication number 356-630, and was filed on 1 October 1986. The Review is published quarterly (four issues annually) with an annual subscription price of $8.75 (sold by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402). The complete mailing address of the office of publication is: Scientific Publications Office, F/NWR1, NMFS, NOAA, 7600 Sand Point Way N.E., Bin C15700, Seattle, WA 98115. The complete mailing address of the headquarters of the publishing agency is: National Marine Fisheries Service, NOAA, Department of Commerce, 2001 Wisconsin Ave., N.W., Washington, DC 20235. The name of the publisher is Jack McCormick and the editor and managing editor is Willis Hobert; their mailing address is: NMFS Scientific Publications Office, 7600 Sand Point Way N.E., Bin C15700, Seattle, WA 98115. The owner is the U.S. Department of Commerce, 14th St., N.W., Washington, DC 20230; there are no bondholders, mortgagees, or other security holders. The purpose, function, and nonprofit status of the organization (agency) and the exempt status for Federal income tax purposes has not changed during the preceding 12 months. The extent and nature of circulation is as follows: Total number of copies (A) (average number of copies of each issue during the preceding 12 months) was 2,400 and the actual number of copies of the single issue published nearest to the filing date was 2,400. Paid circulation (B) is handled by the U.S. Government Printing Office, Washington, DC 20402, and (C) the total number printed for their sales (mail subscriptions and individual sales) was 730 for both the average number of copies each issue during the preceding 12 months and the actual number of copies of the single issue published nearest to the filing date. Free distribution (D) by mail, carrier, or other means; samples, complimentary, and other free copies (average number of copies each issue during the preceding 12 months) was 1,030 and the actual number of copies of the single issue published nearest to the filing date was 1,650. The total distribution (E: sum of C and D) (average number of copies each issue during the preceding 12 months) was 2,400 and the actual number of copies of the single issue published nearest to the filing date was 2,400. There were no copies not distributed or returned from news agents (F). The total (G: sum of E and F) is equal to the net press run figures shown in item A: 2,400 and 2,400 copies, respectively. I certify that the statements made by me above are correct and complete: (Signed) Jack McCormick, Publisher.

NMFS Helps to Preserve Louisiana Wetlands, Fish

The National Oceanic and Atmospheric Administration (NOAA) is helping protect economically vital fish habitat in a Federal-state effort to recover valuable Louisiana coastal wetlands. Biologists with NOAA's National Marine Fisheries Service have developed procedures for the operation of water control structures installed in Louisiana canals to allow fish to enter the marshlands during crucial early life stages.

In 1985, 27 percent of the 6.3 billion pounds of fish harvested in the U.S. were caught off Louisiana. The state's catch that year was worth more than $229 million at dockside. About 90 percent of those fish, including shrimp, menhaden, seatrout, croaker, flounder, and blue crabs, depend on wetlands during some stage of their development. For the past 3 years, Louisiana has been losing about 32,000 acres of coastal land annually.

NMFS biologists at Galveston, Tex., have reviewed plans for about 300,000 acres of wetlands and for the last 3 years have encouraged land managers to control the structures to allow young fish and shellfish to enter the marsh area and return to the Gulf of Mexico after maturing. Land managers used the structures to increase the water level in ponds by the start of the waterfowl hunting season in November to provide better marsh habitat for ducks and geese that migrate to the area during the winter. In the spring and summer they would drain the marshes to allow new plant growth. However, this method prevented several important species of fish and shellfish from entering the marsh area during their peak migratory periods.

The Commerce Department agency has provided guidance for the management of about 200 of the structures installed throughout the state's 2.5 million acres of marshlands. They are installed across man-made canals to alleviate the saltwater intrusion and extreme water levels that often damage plant life.

The canals are built to make the marshland accessible to barges for oil and gas exploration. The control structures regulate the direction, velocity and amount of fresh and salt water allowed to enter or leave the canals. The wetland conservation effort involves marsh owners, the NMFS, Soil Conservation Service, Fish and Wildlife Service, Corps of Engineers, and Louisiana's Department of Wildlife and Fisheries and Natural Resources.

FDA, NMFS, SIGN MOU

The U.S. Food and Drug Administration (FDA) has provided notice of a memorandum of understanding (MOU) between the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and the FDA of the Public Health Service, U.S. Department of Health and Human Services. This MOU describes the cooperative methods that FDA and NMFS will employ to deal with illegal commerce in molluscan shellfish. The agreement became effective on 24 July 1986 and further information on it is available from: Walter J. Kustka, Intergovernmental and Industry Affairs Staff (HFC-50), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, (301) 443-1583. (Source: 5IFR, No. 164, 30271.)

Shrimp Culture Is Promoted in Burma

The Burmese Government is promoting a shrimp culture industry. The state-owned People's Pearl and Fishery Corporation (PPFC), Burma's sole shrimp farming and exporting organization, has built some ponds and more are planned. The PPFC also plans to build a hatchery. Government officials realize the potential value of cultured shrimp as a means of increasing export revenues and foreign currency earnings. Current efforts have concentrated on freshwater shrimp and the first harvest was reported in 1984. The PPFC is currently conducting experiments with marine species and plans to culture large quantities of marine shrimp (Table 1).

Species and Grounds

Approximately 25 shrimp species are known to exist in Burma. Shrimp are harvested offshore by the commercial trawler fleet and in shallow coastal areas by artisanal fishermen. Yellow shrimp, *Metapenaeus brevicornis*, and speckled shrimp, *M. monoceros*, are the main offshore species caught, although some penaeid species are also harvested by commercial trawlermen. Giant tiger prawn, *Penaeus monodon*; banana prawn, *P. merguiensis*, and redtail prawn, *P. penicillatus*, predominate in the artisanal catches, but small quantities of *Metapenaeus* spp. are also caught by artisanal fishermen. The PPFC, as the result of studies conducted in the late 1970's and early 1980's, identified two particularly promising species. The most promising marine species was *P. monodon* and the most successful freshwater species was the giant river prawn *Macrobrachium rosenbergii*.

The Irrawaddy River Delta area, comprised of swamps, mangroves, and other estuaries, offers the best freshwater, brackish water, and marine culture sites. Burma's other coastal regions (the Rakhine coast in the west and the Tenasserim coast, bordering on Thailand, in the east), also have substantial wild shrimp resources. Mining and the utilization of mangroves for charcoal production, however, are destroying the estuatine habitat of postlarval shrimp and polluting potential shrimp culture sites.

Farms and Technology

The PPFC's major emphasis has been on freshwater shrimp. The first successful grow-out studies on *M. rosenbergii* were completed in 1979 at the Thaketa Research Station in the Rangoon District. The Thekata Station is now Burma's main hatchery for fresh water prawn postlarvae. The hatchery technology used by the PPFC is based on the "clearwater" method. This involves the constant filtration and aeration of water in larval tanks to maintain appropriate temperature, oxygen, and salinity levels, and to eliminate metabolic wastes. This method, which is used in marine, freshwater, and brackishwater shrimp culture, is distinguished from the "greenwater" method, a freshwater culture technique that relies on phytoplankton culture in tanks to control ammonia build up. The "clearwater" hatchery method is the most common technique used in Asia. Zooplankton, mainly *Artemia*, and phytoplankton are cultured to feed the larval shrimp.

The PPFC operates a semi-intensive farm for *M. rosenbergii* production at Thanatpin. Semi-intensive culture techniques consist of moderate water exchange rates (less than 100 percent per day) in culture ponds. Cultured organic foods (for example, phytoplankton), stimulated by fertilizers, are the primary source of food. To supplement natural foods, cooked fish by-products or coarse grains are added to the ponds. Stocking densities, exceeding 30,000 postlarvae per hectare, also characterize semi-intensive culture. In countries like Burma, farmers using semi-intensive techniques, obtain postlarvae from the wild, but hatchery construction will soon enable farmers to acquire cultured postlarvae.

The circulation of water for the Thanatpin farm's earthen-walled ponds is controlled with sluice gates and a pump system, respectively. Fertilizer, usually manure, is added to the ponds to stimulate natural food growth, although rice bran and oil cake may sometimes be placed in ponds to supplement natural foods. The 40 hectare (ha) Thanatpin farm is expected to achieve yields of 50 t of shrimp a year when operating at full capacity.

The PPFC is also researching marine shrimp species. Experiments are underway at Naukme (Irrawaddy District) and Sandoway (Rakhine District), to raise *P. monodon* by utilizing extensive methods. In Asia, farmers utilizing extensive culture methods often rely on wild postlarval shrimp entering culture sites on incoming tides or during flooding. Depending on the type and location of the culture site, farmers may use irrigation systems and sluice gates to control water flow to the sites. In some instances, wild postlarvae are collected by hand from other sources (rivers, lakes, etc.) and then stocked by farmers in

Table 1.—Important shrimps in Burma[1].

Scientific name	English name	Spanish name	French name
Macrobrachium rosenbergii	Giant river prawn	Camaron gigante	Bouquet geant
Metapenaeus brevicornis	Yellow shrimp	Camaron amarillo	Crevette jaune
Metapenaeus monoceros	Speckled shrimp	Camaron moteado	Crevette mouchetee
Penaeus merguiensis	Banana prawn	Camaron banana	Crevette banana
Penaeus monodon	Giant tiger prawn	Camaron tigre gigante	Crevette geante tigree
Penaeus penicillatus	Redtail prawn	Camaron rabo colorado	Crevette queue rouge

[1]Source: Holthuis, L. B. FAO Species Catalogue: Shrimps and Prawns of the World. FIR/s125 Vol. 1.

culture sites. Farmers do not use feeds or fertilizers and rely on the natural productivity of the culture site for the survival and growth of postlarvae.

The Naukme and Sandoway farmers use passive stocking methods, such as allowing tidal flow to wash postlarvae into culture sites. However, they also occasionally purchase postlarval shrimp collected from nearby estuaries.

Burma's PPFC presently has only one other 50 ha freshwater farm (location unknown) in addition to the 40 ha farm at Thanatpin. The Burmese Government plans to construct an additional four freshwater farms of 50 ha each by 1990, for a total of six farms. While this expansion plan appears feasible, the Burmese Government's goal of having 4,000 ha of marine culture farms operational by 1990—in addition to the 300 ha of freshwater farms mentioned above—seems somewhat optimistic.

Development Projects

Burma's $43-million Inland Fisheries Development Project has two shrimp components. The first is aimed at constructing freshwater and marine shrimp farms with supporting hatcheries. The second part is designed to improve the quality of both wild and cultured shrimp catch by providing better nets and fishing gear to artisanal fishermen, improving the vessels for collecting the shrimp harvest in outlying regions, constructing new collection stations, and expanding a fishery processing plant in Rangoon. To prevent spoilage, ice plants will also be constructed to supply collection stations and transportation vehicles. At present, inadequate collection infrastructure results in considerable spoilage before the catch can be processed. The Asian Development Bank will loan the Burmese Government $20 million for the Project, while the remaining $23 million will be financed by Burma. In discussing the guidelines for Burma's Fifth Four-Year Plan at the Fifth Party Congress, party and state officials emphasized that their goal in promoting a shrimp culture industry is to increase exports.

Shrimp Catch

Burma's shrimp catch increased from 5,155 t in 1984 to 6,931 t in 1985, or by almost 1,800 t (34 percent) (Table 2). Alleged overfishing has already caused some observers to believe that the Burmese shrimp catch will decline in the future. This is based on the decreasing size of the shrimp landed in Burma. The PPFC, however, expects the shrimp catch to double by 1990 to 13,490 t.

Cultured Shrimp

Burma first produced cultured shrimp in 1984, when 7 t of freshwater prawns was harvested. In 1985, about 10 t was harvested. The Government plans a major expansion of the industry and forecasts that harvests of cultured shrimp will reach 1,240 t by 1990. Of this total, 420 t would be freshwater species and 820 t marine species. In calculating these forecasts, the Burmese Government expects shrimp yields on freshwater farms to average 1.4 t (whole weight) per ha per year, while on marine farms, where extensive methods will be used, shrimp yields are expected to average only 0.2 t per ha per year.

Table 2.—Burma's shrimp production: Cultured, wild, and total, 1983-90.

	Catch[1] (t)			
	Aquaculture			
Year	Marine	Freshwater	Wild[2]	Total
1983			4,356.0	4,356.0
1984		7	5,148.2	5,155.2
1985		10	6,921.4	6,931.4
1990[3]	820	420	12,250.0	13,490.0

[1]The catch is given in whole weight (heads on).
[2]Does not include unknown quantities of metapenaeid shrimp which is not purchased by the PPFC.
[3]Projected.

Wild Shrimp

More than half of Burma's wild shrimp catch is harvested by artisanal fishermen. Penaeid species (the only species the PPFC purchases, processes, and exports) amount to 80-82 perccent of the total artisanal shrimp catch. Recent surveys indicate that offshore trawler catch rates of 30-60 kg/hour are attained off Burma's coasts. The highest rates have been recorded just after the monsoon season ends. However, since a large portion of the offshore commercial trawler catch consists of metapenaeid shrimp, a species not purchased by the PPFC, the trawler fleet is forced to either sell its metapenaeid catch to neighboring Thai fishermen, or to local Burmese consumers. Such over-the-side or local sales are usually not reported to the PPFC whose statistics include only the amont of penaeid shrimp species it purchases itself. As a result, the actual amount of Burma's wild shrimp catch cannot be precisely determined.

Exports

Burma's total shrimp exports more than doubled in 1984 to 2,610 t from 1,190 t in 1983. Data for 1985 were not available (Table 3). By comparison with neighboring countries, such as Thailand, Bangladesh, and India, Burma's exports are small. Most of the exported shrimp is either frozen (headless shell-on) or dried. Burma's main shrimp export markets are traditionally the United States and Japan. In 1985, Burma exported 743 t of shrimp to the United States, almost a 100 percent increase over the 1984 total of 381 t. Total 1985 exports to Japan were 348 t. Because of the demand in Japan for *M. rosenbergii*, Japanese companies have invested $4 million in a Burmese freshwater aquaculture farm designed specifically for export. The farm is expected to produce annual harvests of 450 t of shrimp. (Prior to this project, Burma had established a shrimp trawling joint venture with Japan off the northern Rakhine coast, but it is not yet known if the project was successful.)

Burma, like other Asian shrimp exporting countries, has experienced quality control problems. Poor catch and landing techniques and primitive transportation and processing technology are responsible for the low quality of Burma's shrimp products and the inability of Burmese shrimp exporters to compete on the world markets. A major objective of the Inland Fisheries Development Project is to solve these quality control problems.

Outlook

Despite the Burmese Government's ambitious estimates for rapid growth in its shrimp culture and capture industries, several obstacles may impede this growth. The failure of the Burmese Government to implement adequate management plans to control overfishing may result in rapid depletion of both offshore and artisanal shrimp fisheries. In addition, many potential grounds are being ruined by other forms of natural resource exploitation (i.e., the extraction of mineral and forestry products which pollute and destroy natural shrimp habitats).

Table 3.—Burma's shrimp exports, by country, quantity, and value, 1980-85.

	Exports (t)						Exports (US$1,000)					
Country	1980	1981	1982	1983	1984	1985	1980	1981	1982	1983	1984	1985
U.S.A.	279.5	416.3	1,503.8	296.5	380.8	743.5	2,278	3,363	11,191	1,990	2,763	4,691
Japan	812.8	1,198.5	623.8	487.1	336.4	348.2	4,565	6,307	4,667	3,991	2,699	2,725
EEC	39.0	62.0	63.0	24.0	NA	NA	161	367	394	245	NA	NA
Other	NA	NA	109.4	382.4	NA	NA	NA	NA	NA	NA	NA	NA
Total	NA	NA	2,300.0	1,190.0	2,610.0	NA	NA	NA	NA	NA	10,500	NA

U.S. Tuna Imports From Latin America, 1978-85

U.S. tuna imports from Latin America set an all-time record in 1985. Since 1979, when the previous record was set, shipments had been substantially lower. Frozen and canned tuna imports from Latin America in 1985 totaled 82,000 metric tons (t), valued at $77 million (Fig. 1, Tables 1 and 2). The increased shipments were primarily caused by developments in six Latin American countries and dependencies (Venezuela, Ecuador, Panama, Brazil, the Netherlands Antilles, and the British Virgin Islands), all of which substantially increased their shipments to the United States. The increased imports from these countries more than made up for the continuation of the U.S. tuna embargo on Mexico, which was the principal Latin American supplier to the U.S. market until it seized a U.S. tuna seiner in 1980.

Regional Importance

U.S. imports from Latin America in 1985 accounted for over 25 percent of all U.S. tuna imports. Only shipments from Asian countries (totaling over 180,000 t and including foreign landings in American Samoa) exceeded Latin American shipments (Fig. 2). African and European countries also shipped tuna to the United States: 27,000 t and 18,000 t or 9 and 6 percent, respectively, of all U.S. tuna imports totaling 314,200 t. The expanded 1985 shipments from Latin America substantial-

ly increased the Latin American market share in the United States. Latin America's 25 percent share of all U.S. 1985 tuna imports was nearly double the region's 1984 share of only 13 percent. The increased Latin American market share was also aided by a decline in shipments from Asia.

Commodities and Species

Frozen

Most Latin American countries ship tuna to the United States frozen. About 97 percent of all 1985 tuna imports from Latin America were frozen. The two most important species imported from Latin America were yellowfin and skipjack tuna, but there were also lesser quantities of albacore. Albacore imports from Latin America totaled 6,000 t in 1985. Breakdowns for yellowfin and skipjack tuna are not available because the U.S. Customs Service has several tariff categories that include both species.

Latin American tuna fisheries are generally oriented toward foreign markets. Most countries export a sizeable part of their tuna catch, primarily to the United States for canning. Almost all U.S. imports from Latin America are shipped as whole or eviscerated fish. A small amount is processed as loins and

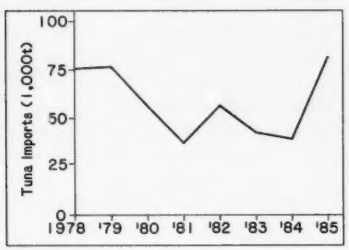

Figure 1.—U.S. tuna imports from Latin America, 1978-85.

Table 1.—U.S. tuna imports from Latin America (all product forms), by quantity, 1978-85.

Country or Dependency	Imports (t)							
	1978	1979	1980	1981	1982	1983	1984	1985
Venezuela	9,324.3	5,066.1	4,062.0	9,970.7	9,615.1	9,357.7	8,082.0	24,678.7
Ecuador	12,535.4	17,134.5	11,845.2		1.8	864.5	6,707.1	19,765.9
Panama	14,519.8	25,684.9	16,201.7	14,297.3	21,695.1	11,294.2	15,795.0	17,758.4
Brazil	708.3	395.0	4,743.3	6,286.4	14,122.1	13,528.5	6,097.9	13,860.8
Neth. Antilles	7,670.9	11,753.9	11,837.0	2,334.5	913.5	137.2	248.4	3,576.6
Brit. Virgin Isl.						15.2		1,021.1
Uruguay	2,654.2	710.8	1,602.9	1,639.1	1,058.9	466.0	538.1	654.3
Dominican Republ.				19.1		445.8	819.1	269.0
Argentina				12.2	45.8	50.8	11.0	89.0
Peru	40.6	86.6	510.4	23.4	137.2	11.4	13.3	39.8
Trinidad-Tobago		12.5	242.2	522.9	19.7	783.8	349.5	33.4
Chile			3.7	25.4	39.3	1.2	43.0	16.0
Costa Rica	573.2	558.0	450.0		787.6	174.6	600.0	0.6
Guyana								0.5
Cayman Islands			294.8	1,908.9	7,833.5	3,910.8	316.1	
Bermuda	6,300.7	4,380.6		445.7	405.7	0.5	1.3	
French West Indies		59.2	14.7	56.8	0.7	195.0	1.2	
Bahamas	529.7					181.6		
Honduras	4.7					1,245.4		
El Salvador					201.4	247.0		
Barbados				68.0				
Mexico	17,853.1	10,038.1	4,730.7					
Nicaragua	2,988.4	846.0						
Total[1]	75,703.3	76,726.2	56,538.7	37,610.4	56,877.3	42,910.9	39,623.1	81,764.0

[1]Totals may not agree due to rounding.

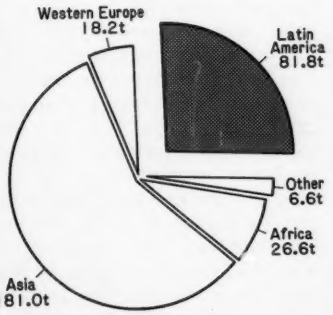

Figure 2.—U.S. imports of tuna by region (total = 314,200 t).

Table 2.—U.S. tuna imports from Latin America (all product forms), by value, 1978-85.

Country or Dependency	Imports (US$1,000)							
	1978	1979	1980	1981	1982	1983	1984	1985
Venezuela	7,858.7	2,711.3	3,068.9	12,793.5	10,940.2	8,708.4	7,357.5	23,905.8
Ecuador	7,941.9	12,186.3	11,136.4		2.3	677.9	5,099.9	16,915.4
Panama	12,605.9	23,410.3	18,799.2	18,270.8	25,832.8	11,130.6	11,756.2	14,704.7
Brazil	448.3	291.4	5,205.9	7,440.8	15,709.2	12,144.6	6,069.7	11,288.3
Neth. Antilles	7,823.9	10,072.5	14,394.8	4,576.0	1,177.4	121.7	473.8	6,436.0
Brit. Virgin Isl.						9.3		1,344.4
Uruguay	4,434.0	985.1	3,383.0	2,749.4	2,264.1	615.9	966.7	1,254.5
Dominican Republ.				35.2		687.3	1,333.4	438.5
Argentina				26.8	92.1	91.2	18.8	202.1
Peru	53.3	107.4	777.6	19.2	277.9	15.5	28.0	84.1
Trinidad-Tobago		21.7	523.0	710.3	35.1	1,053.5	444.9	72.8
Chile			4.4	35.6	65.7	3.3	105.3	68.2
Costa Rica	499.9	464.4	382.5		826.8	131.1	582.0	1.5
Guyana								1.3
Cayman Islands			277.5	2,279.2	9,197.8	3,723.8	256.6	
Bermuda	1,599.5	1,337.3		520.6	449.5	2.4	3.8	
French West Indies		97.3	53.0	124.2	1.1	258.0	1.6	
Bahamas	175.2					269.5		
Honduras	5.6					1,120.5		
El Salvador					181.0	145.0		
Barbados				222.2				
Mexico	16,221.7	9,976.3	5,110.8					
Nicaragua	1,598.4	715.8						
Total[1]	61,266.2	62,377.0	63,117.0	49,803.7	67,052.0	40,909.5	34,498.2	76,717.5

[1]Totals may not agree due to rounding.

discs by low-cost local labor. The amount of tuna shipped as loins and discs has decreased since 1979 when 1,900 t was shipped in this form, mostly from Mexico and Ecuador (Table 3).

The shipments of loins and discs ceased after 1980 when embargoes were placed on Mexico, Ecuador, and Costa Rica following their seizures of U.S. tuna seiners in jurisdictions not recognized by the United States (Table 4). Exports to the United States were resumed in 1984 when small quantities were shipped. Ecuador was the only Latin American country exporting loins and discs to the United States in 1985 with shipments totaling 420 t.

Canned

Several Latin American countries (Mexico, Ecuador, Venezuela, Brazil, and Costa Rica) can tuna, but these countries produce that commodity mainly for their domestic markets. The one exception is Ecuador, which developed export markets for canned tuna in Colombia and Venezuela. Latin American canners have generally had difficulty exporting their products because of quality control problems and high production costs. Canners in most countries have to import canning materials, processing equipment, oil, and other supplies. The cost of these imports substantially increases production costs.

U.S. imports of canned Latin American tuna are small, but are increasing. The United States imported 2,800 t of canned tuna from Latin America in 1985, compared with only 430 t in 1984 and 12 t in 1983 (Table 5). Almost all 1985 shipments were from Ecuador and, to a lesser extent, Venezuela. The Latin American country with the largest tuna canning industry is Mexico but, because of the 1980 tuna embargo, Mexico cannot ship tuna to the United States (Table 4). Data on production costs in the Mexican tuna canning industry are unavailable, but some observers believe that, when and if the embargo is lifted, Mexico could begin to export substantial quantities of canned product to the United States. Mexican companies are believed to have large inventories of canned tuna, but the specific quantities involved are unknown. One unconfirmed report suggests that Mexican inventories could contain as many as 1 million cases. Discussions with Mexico on the removal of the tuna embargo have touched on the possibility of Mexico restraining shipments of these inventories to the United States.

Table 3.—Latin American exports of tuna loins and disks to the United States, 1978-85.

Country or Dependency	Exports (t)			
	1978	1979	1980[1]	1985
Costa Rica	15.2			
Ecuador	6.4	325.7	214.3	421.0
Mexico	1,331.3	1,528.5	575.7	
Neth. Antilles		37.5		
Total[2]	1,352.9	1,891.7	790.0	421.0

[1]No data for 1981-83 and only 0.5 t exported in 1984.
[2]Totals may not agree due to rounding. Source: U.S. Department of Commerce, Bureau of the Census.

Table 4.—U.S. tuna embargoes on Latin American countries, 1976-86.

Country	Effective date	Date rescinded	Statute[1]	Tuna and products
Costa Rica	2-01-80	2-26-82	MFCMA	All
Costa Rica	4-24-86	In force	MFCMA	All
Ecuador	11-21-80	4-19-83	MFCMA	All
Mexico	7-14-80	In force	MFCMA	All
Mexico	2-01-81	5-21-86	MMPA	Yellowfin and tuna products
Peru	1-01-78	7-01-83	MMPA	Yellowfin and tuna products

[1]MFCMA = Magnuson Fisheries Conservation and Management Act, MMPA = Marine Mammal Protection Act.

Table 5.—U.S. canned tuna imports from Latin America, 1983-85.

Country or Dependency	Imports (t)		
	1983	1984	1985
Argentina			39.2
Brazil		12.8	
Chile			4.5
Ecuador		403.8	2,347.2
French West Indies		0.6	
Netherlands Antilles	0.6	2.6	
Peru	11.4	13.3	39.8
Venezuela		1.4	418.8
Total[1]	12.0	434.5	2,849.5

[1]Totals may not agree due to rounding.

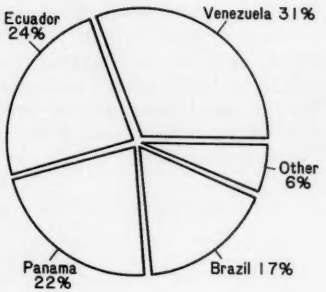

Figure 3.—U.S. tuna imports from Latin America by country, 1985 (total = 81,800 t).

Major Suppliers

Four Latin American countries (Venezuela, Ecuador, Panama, and Brazil) shipped 76,000 t of tuna to the U.S. in 1985, over 93 percent of all tuna shipments from the region (Table 1 and Fig. 3). All four countries have been long-time suppliers of tuna to the United States. Both Venezuelan and Ecuadorean shipments set new records in 1985. Major 1985 developments in these and several other important Latin American countries are listed below.

Venezuela

Venezuela in 1985 became the leading Latin American supplier of tuna to the U.S. market. Shipments totaled 24,700 t in 1985, three times the 8,100 t imported by the U.S. in 1984. Most of the imports from Venezuela were frozen yellowfin tuna, but 1985 shipments also included over 400 t of canned tuna, up from only 1 t in 1984. In the past few years, Venezuela has acquired Latin America's second largest tuna seiner fleet. In 1985, Venezuela operated about 20 purse seiners with a carrying capacity of 22,000 short tons and a small baitboat fleet. Venezuelan companies operate 10 additional foreign-registered seiners in the Eastern Pacific, with a carrying capacity of 11,200 short tons. The Venezuelan fleet was second only to the Mexican fleet. Unlike the Mexican fleet, however, the Venezuelan fleet has been acquired without massive state subsidies and, with the important exception of low-cost fuel, is not currently

subsidized by the Government.

Venezuelan tuna fishermen complain that Government policies, such as those requiring part of their catch to be sold domestically, have actually impeded the industry's development. Many Venezuelan companies have acquired used U.S. vessels. Venezuelan tuna companies are reporting record profits, in part because their seiners were purchased at low prices and because they can buy diesel fuel at prices substantially below international levels.

Venezuelan companies export more than half of their catch and can the rest for domestic consumption. Tuna has replaced sardines as the most popular canned fish product in Venezuela. As part of an agreement signed with the Venezuelan Government in 1983, the country's fishermen are required to land at least 40 percent of their catch in Venezuela to supply the domestic market, leaving only 60 percent for export. (Unconfirmed reports suggest that Venezuelan fishermen underreport their catch in order to reduce the amount of the catch which must be reserved for domestic use and increase the share which can be exported. Venezuelan officials are concerned about the illegal trade and as of April 1986 had reportedly restricted exports to increase the availability of tuna on the domestic market.)

Additional supplies of tuna are received from foreign fishermen who land some of their catch in Venezuela in exchange for the right to purchase diesel fuel below international prices. Some U.S. fishermen have developed successful charter arrangements with Venezuelan companies, but others have reported difficulty with contractual relationships they have attempted in Venezuela. The Venezuelan tuna industry has recovered from a difficult period following the devaluation of the bolivar in 1982 and 1983 when the country's controlled tuna prices fell substantially below international levels. The Government now maintains the controlled domestic price near the world price. Aside from the United States, Venezuela's major tuna market is Western Europe, which imported nearly 14,000 t of Venezuelan tuna in 1984, the latest year for which data are available. Unlike many other Latin American exporters, however, Venezuela has not yet begun to export tuna to Japan.

Ecuador

Ecuador was the second leading tuna supplier in 1985. Ecuadorean companies shipped 20,000 t of tuna to the U.S. market in 1985, almost triple the 6,700 t shipped in 1984. The sharp increase in shipments signifies that Ecuador has finally recovered from the lingering effects of the 1980-1983 U.S. tuna embargo (Table 4). Although the embargo was removed in 1983, export shipments remained well below pre-embargo levels through 1984. Most of Ecuador's 1985 exports were skipjack tuna. In 1985, Ecuador remained the most important Latin American supplier of canned tuna to the U.S. market.

Ecuadorean shipments of canned tuna grew almost 500 percent in 1985, from only 400 t in 1984 to 2,350 in 1985. Ecuador had an active fleet of 30 seiners and 4 baitboats, primarily small vessels. The fleet's carrying capacity in 1985 was only 7,100 short tons. In addition, three U.S.-owned seiners, registered in Vanuatu, fished off Ecuador during 1985. U.S. companies have previously been active in the processing industry, but have now withdrawn. A U.S. tuna company sold its share of the Ecuadorean joint venture INEPACA[1] to local investors in December 1985.

After the United States imposed the tuna embargo in 1980, Ecuadorean tuna companies attempted to market their catch in other Latin American countries. This strategy was partially successful until the region's economic crisis, which developed in 1982 and 1983, forced several countries to impose strict import controls. Some success, however, was achieved and exporters have diversified their markets, shipping canned tuna to Colombia and frozen tuna to various European countries, particularly Spain. Ecuador, like Venezuela, has not yet succeeded in exporting tuna to Japan.

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Panama

Panama was the third leading supplier of tuna to the United States in 1985, shiping 17,800 t, mostly frozen yellowfin tuna. Panama's tuna fleet consisted of six U.S.-owned seiners with a carrying capacity of 7,800 short tons in 1985, but one of these vessels sank. A Panamanian-flag seiner was reportedly the most successful vessel operating in the Eastern Pacific during 1985. Many countries also transship tuna at Panama's Taboquilla Island.

Foreign tuna vessels utilize the port facilities at Balboa, but few use the World Bank-financed fishing port at Vacamonte, which reportedly has not been dredged in recent years. Panama has no domestic tuna-canning industry. Local investors have been considering the construction of a cannery for several years, but declining world tuna prices since 1982 have discouraged such an investment. Like Venezuela, Panama exports significant amounts of tuna to Western Europe—over 12,500 t in 1984; exports to Japan are small.

Brazil

Brazil was the fourth most important supplier of tuna to the United States in 1985. The United States imported 13,900 t of tuna, an increase of 125 percent over 1984. Almost all of Brazil's 1985 shipments were skipjack tuna. While all 1985 U.S. tuna imports from Brazil were frozen, Brazil does have a substantial canning industry which processes tuna for the domestic market. Tuna is reportedly becoming the country's most popular canned fish product. The Brazilian tuna fleet is composed of over 85 small vessels, mainly baitboats, with an increasing number of longliners and seiners.

A Brazilian shipyard, the Companhia Brasileira de Armazenamento, recently launched a seiner with a carrying capacity of 155 t, the largest fishing vessel ever built in Brazil. The major Brazilian tuna grounds are off the country's central and southern coast. The fleet is centered in the port of Itajai. The development of the Brazilian tuna industry has been aided by various joint ventures with Japanese companies, including one with Taiyo Gyogyo. Japan

and Korea (ROK) have been active in the industry. In 1984, Brazilian companies leased 12 foreign-owned vessels. The great majority of Brazil's tuna exports are shipped to the United States, although small quantities are also exported to Western Europe.

Netherlands Antilles and British Virgin Islands

U.S. tuna imports from both the British Virgin Islands and the Netherlands Antilles increased sharply in 1985 from low 1984 levels. The British Virgin Islands, which shipped no tuna to the United States in 1984, provided 1,000 t in 1985. The Netherlands Antilles in 1985 shipped 3,500 t to the United States, up from 200 t in 1984. The Netherlands Antilles shipments were primarily through St. Maartens, a transshipment point (located close to Puerto Rico) that has been active for several years. Frozen albacore was the predominant commodity shipped by both countries. Neither country has a tuna fleet. The tuna exported in 1985 from both countries was probably caught by Korean and Taiwan companies operating longliners, perhaps through local joint ventures. Most of the catch is shipped to Puerto Rico for canning.

Uruguay

U.S. tuna imports from Uruguay totaled about 700 t in 1985, the third year in a row in which shipments have been below 1,000 t. These statistics are difficult to evaluate as U.S. canneries report substantially higher receipts from Uruguay. In 1985, for example, canneries in Puerto Rico reported receiving 8,500 t of tuna from Uruguay. Such statistical anomalies probably result because three Asian countries (Japan, Korea, and Taiwan) operate tuna longliners in the South Atlantic and transship some of their catch in Montevideo.

Unlike other Latin American countries, Uruguay's 1985 exports to the United States were made up entirely of albacore, the most valuable of the three common species shipped by Latin American countries to the United States. In the past few years, Uruguayan joint ventures with Asian countries have begun to operate a few longliners which have been transferred to Uruguayan registry. Various difficulties, however, have plagued these ventures. Some of the vessels involved have fled and Uruguayan authorities have asked INTERPOL to help recover them.

The Asian-registered vessels are not permitted to fish within 200 miles of the coast, which is reserved for Uruguayan vessels. The Asian longliners conduct distant-water operations and land their catch at La Paloma or Montevideo. While officially reported U.S. imports have been declining, exports to Japan have increased. In 1985, Japan imported over 2,100 t of tuna from Uruguay, most of it shipped frozen.

Mexico

Mexico has traditionally been the most important Latin American supplier of tuna to the U.S. market. In 1978, for example, the United States imported over 18,000 t of tuna from Mexico, making it the most important Latin American supplier that year (Table 2). This traditional relationship was, however, interrupted in July 1980, when the United States embargoed tuna imports from Mexico because Mexico seized a U.S. tuna seiner fishing in the Mexican 200-mile Exclusive Economic Zone (Table 4). The United States does not recognize claims by coastal countries to manage highly migratory species like tuna within 200-mile coastal zones. As a result of the embargo on Mexico, there have been no U.S. tuna imports from Mexico since 1980.

The United States is now considering lifting the embargo, but the economics of exporting tuna to the United States have changed. Before the embargo, Mexico had an advantage over all other Latin American countries in that tuna landed at Ensenada, the country's major tuna port, could be inexpensively trucked across the border to nearby canneries in southern California. Most of these canneries, however, are closed and Mexico will have to compete on more equal footing with other Latin American tuna exporters, which ship their catch to canneries in Puerto Rico.

The Mexican tuna industry has had difficulty in adjusting to the loss of the U.S. market, although Mexican officials claim publicly that the U.S. embargo has not hurt the industry. The actual level of Mexican tuna exports is difficult to assess as current tuna export data are not readily available. The Secretaria de Pesca claims that an all-time record of 35,000 t was exported in 1985, mostly to France, Italy, and Japan. The Mexican tuna catch has increased, reaching an all-time record of 85,000 t in 1985, a 33 percent increase over the 64,000 t taken in 1984. Most of the catch is currently marketed in Mexico and the Mexican Government has had to subsidize both the fishing and marketing.

The Mexican fleet has grown dramatically since 1980, and was comprised of 86 vessels with a total capacity of 71,500 short tons in 1985. Most of these recently acquired vessels are modern seiners with carrying capacities of 1,000 short tons or more, capable of distant-water fishing. Most of these purchases were made by private investors taking advantage of heavily subsidized government loans. Mexico's problems in marketing its catch has caused great difficulties for the fishermen and private companies which purchased vessels in 1981 and 1982. For example, about 25 of Mexico's 86 tuna vessels, with a total carrying capacity of over 19,000 short tons (about 25 percent of the fleet), were inactive in 1985. The active vessels are receiving extensive government support.

Most of the Mexican catch is generally yellowfin tuna, but the relative shares of skipjack and yellowfin tuna vary substantially from year to year. The yellowfin tuna catch in 1985 was 79,200 t, an unusually high percentage—nearly 95 percent of the total catch. The success of the yellowfin tuna fishery is important to Mexico because the larger species is easier to export. The high percentage of yellowfin tuna is probably due to the fact that skipjack tuna commands a lower price and is more difficult to export. Mexican fishermen are thus adjusting fishing strategy to target yellowfin tuna, reportedly fishing extensively on porpoise. Fishermen use the porpoise schools to locate yellowfin tuna as the species often schools in association with porpoise. The same relationship, however, does not exist with skipjack tuna. (Source: IFR-86/30.)

The INPFC: Its History and Accomplishments

"Ocean Forum" by Roy I. Jackson and William F. Royce, published by Fishing News Books Ltd., 1 Long Garden Walk, Farnham, Surrey, England, is subtitled "an interpretative history of the International North Pacific Fisheries Commission," known widely as the INPFC and set up by a treaty between Canada, Japan, and the United States.

Concern over Japan's reentry into North American Pacific salmon, halibut, and herring fisheries led to the adoption of the International Convention for the High Seas Fisheries of the North Pacific Ocean in 1953 which established the INPFC to "ensure the maximum sustained productivity of the fishery resources of the North Pacific Ocean." In many ways the INPFC pioneered and excelled in cooperative high-seas marine and anadromous fisheries research, the results of which serve, after peer review, as the basis for fishery management decisions.

The story of how the national governments of Canada, Japan, and the United States came together under the Commission to study, manage, and develop the fisheries is a complex one involving science, business, and politics, with often widely disparate views. At the time, fishery science was still not widely accepted, let alone utilized, as a basis for regulating marine fishing. The concept that marine resources were inexhaustible was still widespread, and the 3-mile limit was the standard for most fishery resource claims.

The authors present and analyze a considerable amount of material in depth. Reviewed are Japanese fisheries before, during, and after World War II, North American apprehensions of the expansion of Japanese fisheries, concerns for fishery resources, and much more. The Convention set up a newly devised principle of Abstention, which set mid-ocean barriers to the eastward expansion of Japan's fisheries, an important aspect of which was that it drew lines on the ocean believed far enough west so Pacific fisheries stocks of concern to North American fishermen would be relatively inaccessible to Japanese fishermen. The concept was innovative at the time and was supposed to protect coastal fishermen from distant-water fishing fleets, provided that the resources were fully utilized, under scientific study, and managed so full harvest would be sustained.

Research and statistical programs had to be undertaken, at first devoted largely to defining the continental origin and ocean distribution of various species of salmon. These are detailed in chapter 4. Chapter 5 reviews Commission problems and problem solving between 1959 and 1965, and chapter 6 covers 1966-77, ending with the effects of the Law of the Sea (LOS) negotiations on the Commission and its work. Chapter 8, "a search for rational fisheries," begins by defining "rational fisheries," and then appraises INPFC contributions, reviews the directions of fishery development, and addresses the future fisheries in the North Pacific and the future role of the INPFC in the region's fishery development.

Ample notes and references are given, and appendices include the 1953 and 1959 Conventions (including memoranda of understanding), a list of INPFC Commissioners and their terms from 1954 to 1985, biographies of some major participants in INPFC research or deliberations, INPFC documents and publications, and excerpts pertaining to fisheries from the Law of the Sea.

The authors call the Commission an "ocean forum" uniquely successful in identifying North Pacific fishery problems and in guiding movement toward solutions and which, they say, should be continued. In sum, the book provides a clear and thoroughly documented account and analysis of the research and accomplishments of a unique body with proven successes in conducting marine fishery research and in fostering high-seas fishery conservation. It should be of considerable interest to historians as well as many who are involved in marine fisheries issues. Indexed, the 240-page hardbound volume is available from the publisher for £19.50 sterling or US$30.00 (surface mail) or £24.00 sterling or US$37.00 (airmail).

Marine Mammals of the N.E. Pacific

Publication of the second edition, revised, of **"Marine Mammals of Eastern North Pacific and Arctic Waters,"** edited by Delphine Haley, has been announced by Pacific Search Press, 222 Dexter Avenue North, Seattle, WA 98109. Updated by recognized authorities (including many active and retired NMFS scientists) on the various marine mammals of the region (including the Bering Sea and Arctic Ocean). Geographical boundaries covered range roughly from Baja California and the Gulf of California (at lat. 22°N) westward to long. 180°, and north to the Arctic Ocean and then eastward to the Alaska-Canada border at long. 140°W. Some of the species covered, of course, may often range well beyond those boundaries.

This new edition considerably updates the data published a decade ago in the first edition. Provided is detailed information on the life history, habitat, range, status, exploitation, and the like for the region's cetaceans, pinnipeds, and other marine-adapted species. Dale Rice covers the blue, gray, sperm (including pygmy and dwarf species), and seven beaked whales (family Ziphiidae). Edward Mitchell covers the fin, sei,

Bryde's and minke whales, while Allen Wolman discusses the humpback whale, James Scarff the right whale, and Willman Marquette the bowhead whale.

Porpoises and dolphins of the region are reviewed by Stephen Leatherwood and Randall Reeves, while Stephen Reilly and Susan Shane cover the pilot whale, Karen Miller and Victor Scheffer the false killer whale, Michael Bigg and Scheffer the killer whale, Francis Fay, the belukha whale, and Murray Newman and Deborah Cavanagh, the narwhal.

Pinniped authors include Clifford Fiscus (northern fur seal), Luis Fleischer (Guadelupe fur seal), Roger Gentry and David Withrow (Steller sea lion), Bruce Mate and Douglas DeMaster (California sea lion), Steven Jeffries and Terrel Newby (Pacific harbor seal), John Burns (ringed, bearded, spotted, and ribbon seals), and Robert DeLong (the northern elephant seal). Karl Kenyon covers the walrus, Hawaiian monk seal, and sea otter, while Jack Lentfer discusses the polar bear, and Delphine Haley covers the Steller sea cow.

In addition, Scheffer provides a chapter on marine mammal conservation and Mitchell has authored a chapter on the origins of the region's sea mammal fauna. An extensive listing of general and specialized references is given, as is the classification of eastern North Pacific marine mammals. The book is amply illustrated with excellent color and black and white photographs and drawings.

Like the first edition, this one is authoritative and is well written, edited, and designed, and both scientists and general readers should find both useful and interesting. Indexed, the paperbound 295-page volume is available from the publisher for $22.95.

Fundamentals of Fisheries Economics

Publication of a revised and enlarged edition of Lee G. Anderson's **"The Economics of Fisheries Management"** has been announced by The Johns Hopkins University Press, 701 West 40th Street, Suite 275, Baltimore, MD 21211. In it, the author has considerably revised and augmented the material in his 1977 volume: New topics include recreational fisheries, fisheries development, and the share system of remuneration.

The author begins with a chapter that introduces the basic principles of economics important to the study of fisheries, and which underpins the rest of the book, especially for those less knowledgeable about economics. The second and third chapters present the main economic analysis and chapter 4 discusses more intricate economic models of fishery exploitation. Chapter 5 moves beyond the analysis of a single fleet harvesting an independent fish stock to several cases that more closely reflect real-world fishery exploitation. Chapter 6 then presents a general discussion of types of fishery regulation and focuses on their economic aspects and on economical ways of implementing them. Finally, chapter 7 provides a brief introduction to some recent empirical studies and shows how the theory of the previous chapters can be used to provide useful information. The 296-page clothbound volume is available from the publisher for $29.95.

Modeling Fisheries and Wildlife Systems

Publication of **"Systems Analysis and Simulation in Wildlife and Fisheries Sciences"** by William E. Grant has been announced by Wiley-Interscience, John Wiley & Sons, Inc., 605 Third Avenue, New York, NY 10158-0012. The author is associate professor in the Department of Wildlife and Fisheries Sciences at Texas A&M University, College Station. The volume differs from some others in that it provides a hands-on approach to teaching modeling skills in the fields of fisheries and wildlife science. Thus, the author provides step-by-step guidelines to the application of systems analysis and simulation to questions about those ecosystems.

He begins by tracing the development of systems ecology, and introduces basic concepts of systems analysis and simulation and illustrates it with two brief case histories of modeling for fisheries, the Gulf of Mexico shrimp fishery. Then follow the four phases of systems analysis: Conceptual model formulation (ch. 2), quantitative specification of the model (ch. 3), model validation (ch. 4), and model use (ch. 5). Within each chapter the various stages associated with the given phase are identified and discussed. First, the theory or concept associated with each step is presented, along with brief examples. Then, the application is demonstrated through a detailed example. The same example, which deals with modeling the growth and harvest of fish in a hypothetical pond system, is carried through each chapter. Chapters 2-5 also provide exercises that deal with modeling the dynamics of a plant-herbivore system; appendix 6 gives the answers to those exercises. The final chapter provides advice on the development and use of models within a management framework.

Other appendices provide selected techniques in matrix algebra and regression analysis, an overview of differential and difference equations and computers, and a computer program listing with which students can develop and use the pond simulation model described in the text. Altogether, this is a clear and useful presentation of the philosophy and techniques of systems analysis and simulation as is applied in the wildlife and fisheries sciences. Hardbound, the 338-page volume is indexed and is available from the publisher for $47.50.

On the Study of Predators and Prey

Research on fishes, amphibians, and reptiles—the "lower" vertebrates—has produced considerable information on predator-prey relationships in recent years. An overview of many of the research advances in several related fields is provided in **"Predator-Prey Relationships: Perspectives and Approaches From the Study of Lower Vertebrates,"** edited by Martin E. Feder and George V. Lauder and published by the University of Chicago Press, 5801 S. Ellis

Ave., Chicago, IL 60637.

The volume resulted from a 1985 symposium held at the joint meeting of the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, and the Society for the Study of Amphibians and Reptiles. Unlike some symposium publications, this one is not a compilation of data or narrowly defined studies. Rather, the editors have attempted to provide a forum for consideration of critical concepts relating to predation from a variety of perspectives and fields. In general, the symposium first considered functional and mechanistic approaches (typically conducted in a laboratory), and then examined the more naturalistic and evolutionary studies usually done in the field.

Topics and authors are: Measuring behavioral energetics, Albert Bennett; neural mechanisms of prey recognition, Gerhard Roth; functional morphology of feeding, Carl Gans; locomotion as related to predator-prey relationships, Paul Webb; a comparative approach to laboratory and field studies in evolutionary biology, Raymond Huey and Bennett; defenses against predators, John Endler; behavioral responses of prey fishes to predators, Gene Helfman; and laboratory and field approaches to the study of adaptation, Stevan Arnold. Another paper, natural history and evolutionary biology by Harry W. Greene, was invited to round out the book, along with the introduction, commentary, and conclusion by the editors.

In stressing both conceptual and practical issues in the analysis of predator-prey relationships, the editors and authors have provided a good review of selected concepts and experimental approaches to the study of predation. The 198-page volume is available from the publisher and costs $26.00 (hardbound) and $11.95 (paperbound).

Marine Pollution and Its Measurement

Publication of **"Marine Pollution"** by R. B. Clark has been announced by the Oxford University Press, 200 Madison Avenue, New York, NY 10016. The author is professor of Zoology at the University of Newcastle upon Tyne and editor of the journal *Marine Pollution Bulletin*.

Concern about marine pollution may range from, on one hand, fear that the sea itself is being destroyed to a belief that the oceans are the ultimate and safest repository for all human-generated waste materials. In this book, the author has attempted to place the elements of marine pollution into perspective to provide basic information needed so that informed judgements can be made on how serious the impacts of certain pollutants may be to the marine environment and to human health, especially.

To assess the effects of wastes on the sea as accurately as possible, one needs to know what types of wastes reach it (and in what quantities), what then happens to them in the sea, what effects they have on the biota there, and finally what threats they pose. And those are the questions that the authors attempts to answer.

In the first chapter he defines pollution and reviews the nature and sources of inputs and the costs of pollution nabatement. Following that are eight chapters that review specific pollutants, their impacts on the marine environment and commercial fisheries, and their threats to human health: Organic wastes, oil spills and clean-up treatments, metals, halogenated hydrocarbons, radioactive materials, and solid wastes and heat (i.e., dredging spoils and industrial solid wastes, plastics, munitions, etc.). Another chapter provides brief overviews on case histories of pollution in specific regions (the North Sea, Baltic Sea, Mediterranean Sea, Caspian Sea, and Caribbean Sea). The final chapter then summarizes and discusses the issues that must be taken into acount in evaluating the impact of pollution and the fundamental scientific questions involved. In addition, the author provides a list of scientifically based reports and reviews for further information. The book is a good and concise review of the probvlems associated with marine pollution. Paperbound, the 215-page volume is available from the publisher for $17.95.

Much larger and more specific is **"Concepts in Marine Pollution Measurements"**, edited by Harris H. White and published by the Maryland Sea Grant College, University of Maryland, 1222 H. J. Patterson Hall, College Park, MD 20742. The 43 papers in it, peer reviewed and edited, examine a wide variety of techniques for measuring marine pollution and its effects on the environment. Many of the contributions point to recent progress in more realistic assessments of the effects of environmental contaminants, and some challenge some of the strategies and techniques used in marine pollution measurement. Overall, the editors challenge environmental researchers to reexamine the fundamental bases of their science, and argue that any useful assessment of pollution impacts depends on holistic conceptual approaches and suites of measurements that require coordinated team planning.

The contributions are arranged under the following general categories: Toxicity tests, laboratory microcosms, community paramenters and measures of community impact, bioaccumulation tests, chemical measurements and effects criteria, anomalies in field specimens, mesocosms and field systems, field monitoring programs, and a summary and synthesis, in which are presented several issues to be considered if environmental science is to develop groundwork for understanding the dynamics of the marine environment and better protect its health. The 743-page clothbound volume is available from the publisher for $12.50.

Pacific Salmonids and Recreational Fisheries

"Marine Recreational Fisheries 10," which is titled **"Recreational Uses, Production and Management of Anadromous Pacific Salmonids,"** and edited by Richard H. Stroud, has been published by the National Coalition for Marine Conservation, Inc., for the International Game Fish Association, the NCMC, and the Sport Fishing Institute. It encompasses the Proceedings of

the Tenth Annual Marine Recreational Fisheries Symposium held 26-27 April 1985 in Seattle, Wash. and chaired by Frank E. Carlton. And as a whole, the book is an excellent survey of the status, biology, fisheries, management and outlook for recreationally important Pacific salmonids, principally the coho, *Oncorhynchus kisutch*, and chinook, *O. tshawytscha*, salmon and sea-run trout of the genus *Salmo*, as well as the challenges facing them today. Also of interest are the discussions of the then-new (March 1985) U.S.-Canada Pacific Salmon Treaty.

The book and the symposium were divided into four Panels: 1) The salmonid resources (with contributions on species biology, environmental factors affecting their abundance, recreational and commercial uses of coho and chinooks, and restoration and enhancement of Pacific salmonids), 2) management for recreational purposes (natural and artificial production, MRF problems in management decisions, and strategy to maximize angler participation), 3) international aspects of stocks and policies (ocean migrations of chinooks, cohos, and steelheads; international problems in and beyond the U.S. FCZ; and consequences to anglers of the new U.S.-Canada Pacific Salmon Treaty), and 4) local and regional aspects of stocks and policies (locations of local and regional salmonid stocks, restoring Columbia Basin salmon under the Northwest Power Act; regional and local problems in salmon and steelhead management; and redirecting the Pacific Northwest's salmon and steelhead management process). A summary and recommendations were made by Peter Larkin. As usual with this series of MRF symposia, contributors and speakers are internationally recognized experts, and it also publishes questions and comments from the floor.

Management of the various stocks of Pacific salmon and trout is often complicated and controversial. Species are often popular as food fish, sport fish, and as treaty-protected species important to Native Americans. But many will find this book to be a good and handy reference, especially for those interested in the recreational fisheries aspects of these species. The 217-page hardbound volume is available from the IGFA, 3000 East Las Olas Boulevard, Ft. Lauderdale, FL 33316 for $15.00.

Problems and Prospects for the Atlantic Salmon

"The Status of the Atlantic Salmon in Scotland" edited by David Jenkins and William M. Shearer, has been published by the Institute of Terrestrial Ecology (ITE), Monks Wood Experimental Station, Abbots Ripton, Huntingdon PE17 2LS England. The volume presents 15 papers from the ITE Symposium 15 at Banchory Research Station, 13-14 February 1985, which, in sum, thoroughly describes the regulation of the fishery (early history to present), the biology of *Salmo salar*, fishing methods, catch statistics, and compares the status of Scotland's salmonids with that of the stocks of other nations.

Of interest are the discussions of 30 years data on exploitation of the species in Scottish waters, changes in fishing methods and gears, management of a rod-and-line and a commercial fishery, effects of the competition of farmed salmon in the market place on the status of commercial salmon fisheries, salmon farming and the future of Atlantic salmon, potential impact of fish culture on wild stocks of Atlantic salmon in Scotland, data and analysis of Norwegian and Irish salmon catches and other papers.

Like other salmon researchers, contributors bemoan gaps in the existing data base and the difficulty in filling them. More data is needed on catch statistics, they report, as well as on a better understanding of the species' ecology. Scottish salmon catches have declined from a peak in the late 1960's and early 1970's to a point closer to the levels of the 1950's, yet the available smolt production estimates show no continuous downward trend. Reduced catches, biologists believe, are related more to biological changes than to overfishing, though they also believe that the establishment of NASCO will provide the mechanism for controlling the distant-water high-seas Atlantic salmon fisheries. The 127-page paperbound volume is available from the publishing agency for £7.30.

On the Right to Fish

"Treaties on Trial, the Continuing Controversy Over Northwest Indian Fishing Rights" by Fay G. Cohen has been published by the University of Washington Press, P.O. Box C-50096, Seattle, WA 98145-0096. Cohen is Associate Professor, Institute for Resource and Environmental Studies, Dalhousie University, Halifax, Nova Scotia, Canada. The book basically updates and extends material published in "Uncommon Controversy" by the U.W. Press on the problems involved in the long-running conflict over Native American fishing rights in Washington, and is told essentially from the tribes point of view. Since similar controversies are found in many other states nationwide, the book will be of interest beyond the Pacific Northwest.

While the original volume (reprinted four times) dealt with three tribes—the Muckleshoots, Puyallups, and Nisquallies—this new volume extends the scope of inquiry to all Northwest tribes engaged in treaty-protected salmon fishing in western Washington and along the Columbia River. Reviewed are historic Indian salmon fisheries, Indian treaties and court decisions impinging on or clarifying them, the problems or controversies involved in leading up to those court decisions, and the "Boldt Decision" in which Federal Judge George Boldt ruled that the Indians were treaty-bound to get half the salmon resource covered by the treaty, and which was eventually upheld by the U.S. Supreme Court. The 256-page book is a report prepared for the American Friends Service Committee, which has been involved activeiy in treaty rights struggles for many years, and is available from the publisher for $20.00 (cloth) and $9.95 (paperbound).

ICLARM Reports Useful for Aquaculturists

Publication of **"A Hatchery Manual for the Common, Chinese and Indian**

Major Carps," by V. G. Jhingran and R. S. V. Pullin, has been announced by ICLARM, the International Center for Living Aquatic Resources Management, MCC P.O. Box 1501, Makati, Metro Manila, Philippines. Number 11 in the ICLARM Studies and Reviews series, the volume begins with brief reviews of the biology of the following cultured carps: Grass carp, *Ctenopharngodon idella*; silver carp, *Hypophthalmichthys molitrix*; bighead carp, *Aristichthys nobilis*; common carp, *Cyprinus carpio*; catla, *Catla catla*; rohu, *Labeo rohita*; and mrigal, *Cirrhinus mrigala*.

Chapter 2 discusses the components of a carp hatchery, including site selection, construction, ponds, and operational procedures. Chapters 3 and 4 outline proper broodfish selection and care and induced spawning and breeding of the common carp, while chapter 6 reviews the transport of live fish seed and broodfish. Chapter 5 provides data on postlarvae and fry rearing, fish feeding, and rearing pond management, and chapter 7 reviews applied genetics of cultured carps.

Carp nutrition and diseases are also thoroughly discussed, and a later chapter provides an outline for routine upkeep and maintenance tasks for carp hatcheries. In addition, specialized scientific equipment and implements needed for a hatchery are detailed, along with requirements for applied research programs in carp culture. Extensive references and additional sources of information are appended, along with selected Asian units of measure and their metric equivalents. The hardbound 191-page volume includes geographical and subject indexes and is a thorough reference for those interested in carp culture. It is available from the publisher or, in the Americas from ISBS, the International Specialized Book Services, Inc., P.O. Box 1632, Beaverton, OR 97075. Owing to considerable delays or losses in surface mail, ICLARM also recommends requesting their publication by airmail.

This volume was copublished by the Asian Development Bank, Manila, Philippines. The authors have provided much practical information for carp hatchery workers and managers, as well as data useful beyond simple hatchery work, to include production of fingerlings for stocking in growout ponds.

Number 12 in the same series is **"The Biology and Culture of Marine Bivalve Molluscs of the Genus *Anadara*"** by M. J. Broom. Several species of this genus constitute an important source of protein in many tropical to warm temperate areas; the more important species include *Anadara granosa*, *A. subcrenata*, and *A. broughtoni*. The author reviews the general biology, ecology, population dynamics, reproduction, and culture methods for those and other species. A major impediment to improving the productivity of culture of the species is predation by gastropod drills and starfish, and the author believes that hatchery production—if economical—could overcome the considerable year-to-year variability in spat supplies for some species. The 37-page paperbound review is priced at $6.00; ISBS shipping and handling is $2.25 for the first book and $1.25 for each additional volume.

Also available are ICLARM Conference Proceedings 10 and 12, the former being a **"Summary Report of the PCARRD-ICLARM Workshop on Philippine Tilapia Economics,"** and the latter being **"Philippine Tilapia Economics,"** edited by I. R. Smith, E. B. Torres, and E. O. Tan, a compilation of the complete workshop proceedings. Number 10 basically reproduces the abstracts of the workshop presentations, along with the reports of the four working groups (inputs, lake-based production systems, land-based production systems, and marketing) plus the workshop recommendations. The workshop included an overview of Philippine tilapia farming practices, problems, and prospects, as well as contributions on the economics of private tilapia hatcheries, a cost analysis of a large-scale hatchery producing *Oreochromis niloticus* fingerlings, and a paper reviewing the impact of the adoption of tilapia farming on a small community. Other papers review the economics of various cage culture systems in the Philippines as well as several land-based fish-pond and rice-fish culture systems, and the transfer of fish culture technology. Five final papers discuss aspects of Philippine tilapia marketing. Conference Proceedings 10, 45 pages and paperbound, is currently out of print; Conference Proceedings, Number 12, 261 pages, paperbound costs $21.00.

ICLARM Technical Reports 14 is **"Experimental Rearing of Nile Tilapia Fry (*Oreochromis niloticus*) for Saltwater Culture"** by Wade O. Watanabe, Ching-Ming Kuo, and Mei-Chan Huang. The study is a preliminary evaluation of several approaches of early salinity exposure for saltwater culture of tilapias. Reproductive performances of the Nile tilapia was monitored under laboratory conditions at various salinities, and the salinity tolerance of the progeny was determined. Survivorship of fertilized eggs, spawned in freshwater but removed from the mouth of the parent female and artifically incubated at various salinities was also evaluated, and the salinity tolerance of resultant fry was determined. The salinity tolerance of fry spawned and hatched in freshwater, but subsequently acclimatized to various salinities was also determined. The 28-page paperbound report costs $6.00.

ICLARM Technical Reports 16 is **"Salinity Tolerance of the Tilapias *Oreochromis aureus*, *O. niloticus* and an *O. mossambicus* × *O. niloticus* hybrid"** by Wade O. Watanabe, Ching-Ming Kuo, and Mei-Chan Huang. In it the authors have evaluated several indices as practical measures of salinity tolerance in the tilapias spawned and reared in freshwater, and evaluated the changes with respect to age, size, and condition factors, and suggested avenues for future research. The 22-page paperbound report costs $6.00.

ICLARM Technical Reports 13 is **"An Atlas of the Growth, Mortality and Recruitment of Philippine Fishes"** by Jose Ingles and Daniel Pauly. The atlas presents the results of a detailed analysis of length-frequency data collected from 1957 to 1981 throughout the Philippines, for 23 families, including 34 genera and 56 species representing 112 stocks of commercially exploited teleosts. The raw data, covering 0.9 million single fish measurements, were compiled by the authors from a number of different sources, particularly

the files of the Philippine Bureau of Fisheries and Aquatic Resources.

The atlas presents estimates of the vital statistics of commercially important Philippine fishes, obtained exclusively through analysis of length-frequency data. The authors have attempted to present information relevant to the fisheries management of tropical fish using the type of data that is most commonly collected from tropical fisheries, i.e., length-frequency data.

The results are presented in the form of 112 plates, providing for each stock: 1) An outline drawing of the fish discussed, with name and sampling location and date, a graphic representation of the length-frequency data with superimposed growth curve, a length-converted catch curve as used for estimating total mortality, a recruitment pattern used to infer the seasonality of spawning and recruitment, and a graph where probabilities of capture are plotted against length to estimate mean length at first capture (L_c). Also, a legend is provided giving, for each stock, the numerical values of the estimates of growth, mortality, and exploitation rate, the source of the data used, brief comments on the biology of the fish in question, and reference to a source of further information.

The aim of this atlas is to provide parameter estimates for the assessment of multispecies stocks in Southeast Asian countries, where most of the species considered are important components of the fishery resource. The growth and mortality parameters estimated here are sufficient for performing single-species yield-per-recruit analyses which may be combined into a multispecies analysis. The authors also suggest using atlas data in the construction of ecosystem models, which usually require parameter estimates for a large number of species. The 127-page paperbound report costs $10.00.

Also published is ICLARM Bibliographies 5, **"A Bibliography of the Giant Clams (Bivalvia:Tridacnidae)"** by J. L. Munro and W. J. Nash. With giant clams garnering interest by aquaculturists, the authors have tried to locate and list all scientific papers and reports published up to 1985 which deal specifically with aspects of the biology, ecology, exploitation, and cultivation of the various species of giant clams. In addition, they have included a selection of papers on topics which are highly relevant to giant clam culture, such as the use of serotonin for spawning induction and key papers dealing with aspects of the biology of the symbiotic zooxanthellae. Omitted were such reports as faunal surveys which merely mention the presence of tridacnids without further detail. The 26-page paperbound bibliography costs $4.50 (airmail) and $2.50 (surface).

Identification of Fish Subpopulations, Remote Sensing, and Fish Hearts

"Applications of Remote Sensing to Fisheries and Coastal Resources," subtitled "Report of a California Sea Grant Workshop" and edited by Rosemary Amidei, has been published by the California Sea Grant College Program, University of California, A-032, La Jolla, CA 92093, as Publ. no. T-CSGCP-012.

This free 68-page paperback is a report on a national workshop sponsored by the California Sea Grant College Program in November 1983, presenting discussions of the status of remote sensing applications to fisheries and coastal resources, operational aspects of remote sensing, and future applicatons of remote sensing.

Also available from the UCSGP is **"Identifying Fish Subpopulations,"** the "Proceedings of a California Sea Grant Workshop," edited by Dennis Hedgecock, Publ. no. T-CSGCP-013. The workshop was held to 1) determine why and how techniques such as electrophoresis are being applied to problems of fisheries research, 2) assess how data on fish subpopulations are presently applied to management policy, and 3) identify future applications of subpopulation identification to fisheries management. Part I includes discussions of the application of electrophoresis to fisheries research and management and Part II is a panel discussion on the usefulness of the "stock concept" in fisheries management. The 51-page paperbound report is also free.

"Social Consequences of Maritime Technological Change" by Alastair Couper is another in the University of Washington's McKernan Lecture series. The author, head of the department of maritime studies at the Institute of Science and Technology, Cardiff, Wales, concentrates primarily on current and future technological changes in ports and shipping, but also includes remarks on the effects of change on commercial fisheries and fishing communities. The 17-page paperbound report, WSG 85-6, costs $3.00 and is available from Washington Sea Grant Communications, 3716 Brooklyn Avenue, N.E., Seattle, WA 98105.

Also published by the UW Sea Grant Program is WSG 85-5, **"Preservation of U.S. Maritime Freedoms: Mission Impossible?"** by Bruce Harlow. In it, the author, a retired U.S. Navy Rear Admiral, outlines his reasons for optimism on navigational rights even though the United States has not signed the LOS treaty, and concludes that the 1982 convention, however imperfect, represents the best codification of the law of the sea available. The 34-page paperbound booklet also costs $3.00.

"Morphology and Innervation of the Fish Heart" by Robert M. Santer is the first book devoted to the subject and is a thorough review of the literature on the structure and development of the fish heart, amplified by the author's extensive observations on the hearts of many species taken from both marine and freshwater habitats. It describes the variations that exist in the structure and innervation of the fish heart, and points out areas needing further investigation. Well illustrated with photomicrographs and drawings, the 102-page paperbound volume is number 89 in the Series "Advances in Anatomy, Embryology, and Cell Biology" published by Springer-Verlag New York, Inc., 44 Hartz Way, Secaucus, NJ 07094-2491 and costs $24.00.

Authors, Titles, and Subjects in the *Marine Fisheries Review* 48(1-4), 1986

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